

Utah State University

DigitalCommons@USU

All Graduate Theses and Dissertations

Graduate Studies

5-2010

Evaluating Native Wheatgrasses for Restoration of Sagebrush Steppes

Jayanti Ray Mukherjee
Utah State University

Follow this and additional works at: <https://digitalcommons.usu.edu/etd>



Part of the [Agricultural and Resource Economics Commons](#), [Ecology and Evolutionary Biology Commons](#), and the [Plant Sciences Commons](#)

Recommended Citation

Mukherjee, Jayanti Ray, "Evaluating Native Wheatgrasses for Restoration of Sagebrush Steppes" (2010). *All Graduate Theses and Dissertations*. 659.
<https://digitalcommons.usu.edu/etd/659>

This Dissertation is brought to you for free and open access by the Graduate Studies at DigitalCommons@USU. It has been accepted for inclusion in All Graduate Theses and Dissertations by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.



EVALUATING NATIVE WHEATGRASSES FOR RESTORATION
OF SAGEBRUSH STEPPES

by

Jayanti Ray Mukherjee

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Ecology

Approved:

Peter B. Adler
Co-Major Professor

Thomas A. Jones
Co-Major Professor

Thomas A. Monaco
Committee Member

Ronald J. Ryel
Committee Member

Christopher A. Call
Committee Member

Byron R. Burnham
Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2010

Copyright © Jayanti Ray Mukherjee 2010

All Rights Reserved

ABSTRACT

Evaluating Native Wheatgrasses for Restoration of Sagebrush Steppes

by

Jayanti Ray Mukherjee, Doctor of Philosophy

Utah State University, 2010

Major Professors: Peter B. Adler & Thomas A. Jones

Department: Wildland Resources

Pseudoroegneria spicata and *Elymus wawawaiensis* are two native perennial bunchgrasses of North America's Intermountain West. Frequent drought, past overgrazing practices, subsequent weed invasions, and increased wildfire frequency have combined to severely degrade natural landscapes in the region, leading to a decline in the abundance of native vegetation. Being formerly widespread throughout the region, *P. spicata* is a favorite for restoration purposes in the Intermountain West. *Elymus wawawaiensis*, which occupies a more restricted distribution in the Intermountain West, is often used as a restoration surrogate for *P. spicata*. However, since most restoration sites are outside the native range of *E. wawawaiensis* and as the use of native plant material may be more desirable than a surrogate, the use of *E. wawawaiensis* as a restoration plant material has been somewhat controversial. The main goal of my research was to identify plant materials of these species with superior seedling growth, drought tolerance, and defoliation tolerance, traits that may contribute to enhanced ecological function in restored rangeland plant communities.

I conducted a growth-chamber study to evaluate morphological and growth-related traits of germinating seedlings of these two species. My study suggested that,

while the two bunchgrasses are similar in many ways, they display fundamentally different strategies at the very-young seedling stage. While *P. spicata* exhibited greater shoot and root biomass to enhance establishment, *E. wawawaiensis* displayed high specific leaf area (SLA) and specific root length (SRL), two traits commonly associated with faster growth.

According to the eco-physiology literature, plants with greater stress tolerance display lesser growth potential. However, my greenhouse study showed that *E. wawawaiensis* was relatively more drought tolerant than *P. spicata*, despite higher expression of growth-related traits, e.g., SLA and SRL. While the two species displayed similar water use efficiency when water was abundant, *E. wawawaiensis* was also more efficient in its water use when drought stress was imposed.

In a field study, I found *E. wawawaiensis* to be twice as defoliation tolerant as *P. spicata*. This study showed that *P. spicata* is typically more productive in the absence of defoliation, but *E. wawawaiensis* was more productive after defoliation due to its superior ability to recover and hence is a better candidate for rangelands that will be grazed.

Hence, my study showed that *E. wawawaiensis*, despite being regarded as a surrogate for *P. spicata*, exhibits superior seedling establishment, drought tolerance, and defoliation tolerance. Therefore, *E. wawawaiensis* has advantages as a restoration species for the Intermountain West.

This research is dedicated to the loving memories of my father...

Lt. Shri Dinesh Chandra Ray

and

to my loving mother (Smt. Abha Ray)

and sister (Sarbari Ray)

ACKNOWLEDGMENTS

Throughout my stay in Logan, there have been many people who have contributed in some way or other to this project. Here I take few moments to thank them all.

I extend my heartiest thankfulness to the USDA for providing financial support for this research. I am deeply indebted to Utah State University for providing me with an intellectually stimulating environment for research and a wonderful and encouraging committee to work with.

Thank You Chris! - for being on my committee, for being so intellectually critical and impeccably understanding and above all just for being out there in those dire hours of need. Thanks Ron! - for always being so encouraging and supportive and also for keeping all of us in high spirits. I really wish I had your eye for detail. Thanks Tom (M)! - for always greeting me with a smile and for passing on to me your “This too shall pass” attitude. Thanks Tom (J)! – just a word of thanks is inadequate for you. Thanks for supporting me, for helping me with my field work, and for being so patient with me. Thanks to you and Taffy for always making me feel at home. Thanks a lot Peter! - for being such a good teacher and being so patient with me. Your critiques and comments were the best challenges and inspiration I ever had! Your lab meetings, which I thoroughly enjoyed, gave me the best intellectually stimulating environment a student could desire.

Thanks to Dr. Johan Du Toit, the head of the Department of Wildland Resources, for his help and support and the Ecology Center for providing me funds for presenting my work in conferences. Thanks to Drs. Jerry Chatterson and Jack Staub for their

support. I would also like to thank my lab mates, Luke, Joanna, Aldo, and Harmony, for their encouragement, enthusiasm, and fruitful and critical comments.

Thanks a lot Dale! for helping me out in every possible way whenever I needed assistance in field and lab work! I would like to sincerely thank Brian, Matt, Eamonn, Justin, Kevin, Breanne, Devin, Chad, Tren, Jenny, Kim, and Gloria for their technical help! I also wanted to thank Susan Durham for her statistical assistance, Noell Hansen for helping me with my medical insurance, Gaye Griffith, Lana Barr, Marsha Bailey, Cecelia Melder, Jane Hansen, Ilene Ballard, Jon Watterson, Sandy Drake, and Pauline Owen for their help!

On a more personal level, I would like to express my heartiest gratitude to my friend Lisa (M) and Bill, for giving me a home away from home. What would I have done without you both! Thanks to my dear friends Mical, Kishor, and Katie. I feel blessed to have people like you around me. My special thanks to my friends Vijayan and Sumanto for being so supportive and encouraging on skype! Thanks also to Yael Lubin, Michal and Udi, Shirli, Amram and Venasa, Ruth, Allen, Asif, Parminder, Linn, Tanushree, Netra, Rohit, Magathi, Urmila, Udit, Nisha, Yan, Amrita, Lisa R, Mary, Brian, Eamonn, and Jacob— all your help means so much to me!

Finally, I want to thank my dad (Lt. Shri Dinesh Chandra Ray), who, I know would have been the happiest person today! Many thanks also to my loving mom (Smt. Abha Ray) and encouraging sister (Sarbari) for sacrificing so much! I thank my family (Prasanta, Aparna, and Chumki) whom I have always taken for granted. Thanks to you, my loving husband (Shomen), without whose support and patience I could not have done this. To you all I am highly indebted!

Jayanti Ray Mukherjee

CONTENTS

	Page
ABSTRACT.	iii
ACKNOWLEDGMENTS.	vi
LIST OF TABLES.	ix
LIST OF FIGURES.	xi
CHAPTER	
1. INTRODUCTION.	1
2. IMMATURE SEEDLING GROWTH OF TWO NORTH AMERICAN NATIVE PERENNIAL BUNCHGRASSES AND THE INVASIVE GRASS <i>BROMUS TECTORUM</i>	10
3. SEED-MASS, SEEDLING MORPHOLOGICAL-TRAIT, AND GROWTH-RATE VARIATION IN <i>PSEUDOROEGNERIA</i> <i>SPICATA</i>	34
4. DROUGHT TOLERANCE IN THE TWO PERENNIAL BUNCHGRASSES WIDELY USED FOR RESTORATION IN THE INTERMOUNTAIN WEST, U.S.A.	63
5. COPING WITH HERBIVORY: RELATIVE RESPONSES OF TWO SEMIARID PERENNIAL BUNCHGRASSES UNDER TWO CLIPPING REGIMES.	91
6. DEFOLIATION RESPONSES OF <i>PSEUDOROEGNERIA</i> <i>SPICATA</i> TO DEFOLIATION AT TWO PLANT DENSITIES. . .	127
7. SYNTHESIS.	155
APPENDIX.	163
CURRICULUM VITAE.	165

LIST OF TABLES

Table		Page
Chapter 1	1. Cultivars (C), germplasms (G), and experimental populations of <i>P. spicata</i> and <i>E. wawawaiensis</i> and their source of origin. . . .	9
Chapter 2	1. <i>F</i> -values for (a) overall analysis of variance for 10 seedling traits of three populations of <i>P. spicata</i> , Secar <i>E. wawawaiensis</i> , and <i>B. tectorum</i> and (b) species and temperature x species contrasts for 10 seedling traits	30
Chapter 3	1. Least-square means for two ploidy levels and analysis of variance <i>F</i> -values for 10 seedlings traits of nine populations of <i>P. spicata</i> .	57
	2. Correlations of 10 seedling traits with seed mass and absolute growth rate under low and high temperature regimes.	58
Chapter 4	1. Analysis of variance (<i>F</i> -values) for six morphological and four physiological traits for four <i>P. spicata</i> (PSSP) and two <i>E. wawawaiensis</i> (ELWA) populations for three watering frequencies (WF).	83
Chapter 5	1. (a) Clipping schedule for “same-date” and “same-stage” clipping regime and undefoliated control in 2006, 2007, 2008, and 2009 and (b) mean, minimum, and maximum, dates of heading for the ‘same stage’ and ‘same date’ clipping regimes for five <i>P. spicata</i> and three <i>E. wawawaiensis</i> populations for the first (2007) and second (2008) years of defoliation.	115
	2. Analysis of variance (<i>F</i> -values) for three morphological traits for five <i>P. spicata</i> (PSSP) and three <i>E. wawawaiensis</i> (ELWA) populations measured in the year of establishment (2006). . . .	116
	3. Repeated measures ANOVA (<i>F</i> - values) for shoot biomass, defoliation tolerance, and specific leaf area (SLA) of five <i>P. spicata</i> (PSSP) and three <i>E. wawawaiensis</i> (ELWA) populations in response to spring defoliation under two clipping regimes. . .	117
	4. Analysis of variance (<i>F</i> -values) for three morphological traits of five <i>P. spicata</i> (PSSP) and three <i>E. wawawaiensis</i> (ELWA) populations in response to spring defoliation (2008) under two clipping regimes.	118
Chapter 6	1. <i>F</i> -values of repeated-measures analysis of variance for shoot biomass production, defoliation tolerance, and stomatal conductance for 12 <i>P. spicata</i> populations in response to two	

	defoliation treatments and two plant densities.	x 146
2.	<i>F</i> – values and <i>P</i> – values for ANOVA of shoot biomass per plant at low and high densities for 2006, 2007, and 2008.	147
3.	<i>F</i> - values for analysis of variance of four morphological root traits of <i>P. spicata</i> populations (P) in response to defoliation (DF) and density (DN).	148
Appendix	A1. Average-monthly and total-annual precipitation (mm) for 2006, 2007, 2008, and 2009 recorded at Logan Experimental Farm, Utah.	164

LIST OF FIGURES

Figure		Page
Chapter 2	1. Means and standard errors for days to root initiation of <i>P. spicata</i> (PSSP), Secar <i>E. wawawaiensis</i> (ELWA), and <i>B. tectorum</i> (BRTE) at low and high temperatures. Comparison of means and standard errors for (a) days to root initiation, (b) days to shoot initiation, (c) shoot biomass, (d) root- to-shoot (R:S) biomass ratio, (e) shoot length, (f) root length , and (g) root-to-shoot (R:S) length ratio of three <i>P. spicata</i> (PSSP) populations, Secar <i>E. wawawaiensis</i> (ELWA), and <i>B. tectorum</i> (BRTE) at low and high temperatures.	31
	2. Comparison of means and standard errors for (a) root biomass, (b) specific leaf area (SLA), and (c) specific root length (SRL) of <i>P. spicata</i> (PSSP), <i>E. wawawaiensis</i> (ELWA), and <i>B. tectorum</i> (BRTE) at low and high temperatures.	32
	3. (a) Means and standard errors for absolute growth rate (AGR) of three <i>P. spicata</i> (PSSP) populations, Secar <i>E. wawawaiensis</i> (ELWA), and <i>B. tectorum</i> (BRTE) at low (lower case) and high (upper case) temperatures and (b) means and standard error for relative growth rate (RGR) across temperatures	33
Chapter 3	1. Means and standard deviations for seed mass of nine populations of <i>P. spicata</i>	59
	2. Comparison of means and standard errors for (a) days to root initiation, (b) shoot biomass, (c) root biomass, (d) root: shoot biomass ratio, (e) root length, (f) specific leaf area (SLA), and (g) absolute growth rate (AGR) of nine <i>P. spicata</i> populations. . . .	60
	3. Comparison of means and standard errors for (a) days to shoot initiation, (b) shoot length, (c) R: S length ratio, and (d) specific root length (SRL) of nine <i>P. spicata</i> populations at low and high temperatures.	61
	4. Correlations among nine <i>P. spicata</i> populations for seedling traits (a) across temperatures and (b) at high temperature. . . .	62
Chapter 4	1. Soil-water content at three watering frequencies (WF) through a 12-week greenhouse experiment. Points denote dates that pots were watered to water-holding capacity.	84

2. Means and standard errors for (a) mid-day xylem pressure potential at high (HF), moderate (MF), and low (LF) watering frequencies averaged across six populations, (b) stomatal conductance at three watering frequencies averaged across six populations, and (c) stomatal conductance of four *P. spicata* (PSSP) and two *E. wawawaiensis* (ELWA) populations averaged across three watering frequencies. 85
3. Means and standard errors for (a) shoot biomass of *P. spicata* and *E. wawawaiensis* averaged across their respective populations and high (HF), moderate (MF), and low (LF) watering frequencies (b) shoot biomass of four *P. spicata* and two *E. wawawaiensis* populations averaged across three watering frequencies, (c) root biomass of *P. spicata* and *E. wawawaiensis* averaged across their respective populations and watering frequencies, (d) root biomass of four *P. spicata* and two *E. wawawaiensis* populations at watering frequencies, (e) total biomass of *P. spicata* and *E. wawawaiensis* averaged across their respective populations and watering frequencies, and (f) total biomass of four *P. spicata* and two *E. wawawaiensis* populations at watering frequencies. 86
4. Means and standard errors for R:S biomass ratio for (a) *P. spicata* and *E. wawawaiensis* averaged across their respective populations at high (HF), moderate (MF), and low (LF) watering frequencies, (b) four *P. spicata* populations averaged across three watering frequencies, and (c) two *E. wawawaiensis* populations at HF, MF, and LF. 87
5. Comparison of means and standard errors for (a) specific leaf area (SLA) for *P. spicata* and *E. wawawaiensis* averaged across their respective populations at high (HF), moderate (MF), and low (LF) watering frequencies, (b) SLA for four *P. spicata* and two *E. wawawaiensis* populations averaged across three watering frequencies, (c) specific root length (SRL) for *P. spicata* and *E. wawawaiensis* averaged across their respective populations and three watering frequencies, and (d) SRL of four *P. spicata* and two *E. wawawaiensis* populations averaged across three watering frequencies. 88
6. Means and standard errors for total water use by (a) *P. spicata* and *E. wawawaiensis* averaged across their respective populations and three watering frequencies and (b) four *P. spicata* and two *E. wawawaiensis* populations at high (HF), moderate (MF), and low (LF) watering frequencies. 89

	7. Means and standard errors for precipitation use efficiency (PUE) of (a) high (HF), moderate (MF), and low (LF) watering frequencies averaged across six populations, (b) <i>P. spicata</i> and <i>E. wawawaiensis</i> averaged across their respective populations and three watering frequencies, and (c) four <i>P. spicata</i> and two <i>E. wawawaiensis</i> populations averaged across three watering frequencies.	90
Chapter 5	1. Means and standard errors for (a) annual shoot biomass, (b) tiller number, and (c) specific leaf area (SLA) for five <i>Pseudoroegneria spicata</i> (PSSP) and three <i>Elymus wawawaiensis</i> (ELWA) populations in the year of establishment (2006).	119
	2. Means and standard errors for heading date for five <i>Pseudoroegneria spicata</i> (PSSP) and three <i>Elymus wawawaiensis</i> (ELWA) populations in the first (2007) and second (2008) years of spring defoliation.	120
	3. Mean annual shoot biomass for control and spring defoliation (applied in 2007 and 2008) treatments for five <i>Pseudoroegneria spicata</i> (PSSP) and three <i>Elymus wawawaiensis</i> (ELWA) populations.	121
	4. Means and standard errors for annual shoot biomass of five <i>Pseudoroegneria spicata</i> (PSSP) and three <i>Elymus wawawaiensis</i> (ELWA) populations from 2007 to 2009 for (a) control and (b) spring-defoliated treatments.	122
	5. Means and standard errors for (a) defoliation tolerance (%) at two clipping regimes, (b) defoliation tolerance (%) after one (2008) and two (2009) years of defoliation, and (c) specific leaf area (SLA) in May and October (2008) for five <i>Pseudoroegneria spicata</i> (PSSP) and three <i>Elymus wawawaiensis</i> (ELWA) populations.	123
	6. Means and standard errors for five <i>Pseudoroegneria spicata</i> (PSSP) and three <i>Elymus wawawaiensis</i> (ELWA) populations for (a) plant basal area (August 2008) and (b) tiller number (May 2008) for control and spring-defoliation treatments.	124
	7. Means and standard errors under two clipping regimes for (a) tiller regrowth following each defoliation treatment in 2007 and 2008 and (b) tiller regrowth for four <i>P. spicata</i> populations, P-27 <i>P. spicata</i> , and three <i>E. wawawaiensis</i> populations in 2007 and 2008.	125

	8. Correlations of (a) control shoot biomass with control SLA, (b) defoliated shoot biomass with defoliated SLA, (c) control shoot biomass with defoliation tolerance at 'same stage', (d) defoliated control shoot biomass with defoliation tolerance at 'same date', (e) SLA with defoliation tolerance at 'same stage', and (f) SLA with defoliation tolerance at 'same date' across five <i>Pseudoroegneria spicata</i> and three <i>Elymus wawawaiensis</i> populations.	126
Chapter 6	1. Means and standard errors of 12 <i>P. spicata</i> populations for average annual shoot biomass under control and defoliated treatments. . .	149
	2. Means and standard errors of shoot biomass (g plant^{-1}) at low and high densities for 2006 – 2008.	150
	3. Means and standard errors for defoliation tolerance of 12 <i>P. spicata</i> populations across low and high densities calculated from three-year total biomass production.	151
	4. Linear regression of (a) three-year total defoliated shoot biomass (DSB) on control shoot biomass (CSB) and (b) defoliation tolerance (DT) on three-year total control shoot biomass.	152
	5. Means and standard errors of 12 <i>P. spicata</i> populations for root biomass in 2008 at high and low densities.	153
	6. Linear regression of reduction in 2008 root biomass (RB) on reduction in 2008 shoot biomass (SB) for (a) high and (b) low density treatments.	154

CHAPTER 1

INTRODUCTION

The long-term function and stability of arid and semi-arid rangeland ecosystems of the North American Intermountain West have been disrupted on several fronts (Richards et al. 1998). Prior to European settlement in the 1840s, Intermountain West is believed to have been in pristine condition, flourishing with native bunchgrasses (Harris 1967). However, since European settlement, unregulated overgrazing caused a decline in the abundance of native grasses in these moisture-limited shrub-steppe ecosystems (Mack and Thompson 1982). This decline helped to accelerate the spread of the opportunistic, exotic annual species, particularly *Bromus tectorum* L. (downy brome). *Bromus tectorum* may germinate in the fall or early spring when air and soil temperatures are cold, and competition from this weed may limit native perennial grass growth. In spite of cold temperatures, *B. tectorum* is able to elongate roots in the early spring and monopolize early-season resources, ultimately producing large quantities of seed in late spring and completing its life cycle before the onset of summer drought. Its dry flammable biomass increases risk of wildfire and fire frequency on the land it occupies. In the Intermountain West, such changes lead to modification of light availability and water and nutrient balance (Booth et al. 2003). This may affect shifts in species composition (Harris and Wilson 1970; Young and Allen 1997; Adler et al. 2005; Young and Clements 2007), reflected by further declines in native species abundance.

To increase the agricultural productivity of this region, exotic perennial species, e.g., crested wheatgrass (*Agropyron desertorum*, *A. cristatum*), were introduced in the late 1800s from Eurasia (Monsen et al. 2004). Crested wheatgrass displayed better

establishment characteristics and superior drought and grazing tolerance compared to the native wheatgrasses, leading to their replacement with this exotic. Repairing damaged wildlands using native species has been a priority since the 1980s (Whisenant 1999), but their inherent lack of grazing tolerance (Caldwell et al. 1981) and their failure to establish in rangelands infested with annual weeds (Asay et al. 2001; Young and Allen 1997) limit the success of native grasses in seedings within the region. Improved native plant materials have demonstrated the potential for increased genetic diversity, improved habitat and grazing resources for livestock and wildlife, and protection of watershed and soil resources (Asay et al. 2001). Therefore, choosing high-performance native plant materials is crucial for decision-making in restoration practice (Jones 2003).

Pseudoroegneria spicata [Pursh] A. Löve (bluebunch wheatgrass), a widely occurring native, C₃, perennial Triticeae bunchgrass, provides high-quality forage (Miller et al. 1986) and is favored for restoration applications. This species is widespread in the semi-arid temperate portions of the 11 western states of the continental US. It is generally considered to be a climax species with wide ecological amplitude across many different plant associations (Miller et al. 1986). *Pseudoroegneria spicata* is predominantly diploid ($2n = 14$) and occasionally autotetraploid ($2n = 28$) (Jensen et al. 1995). Genetic variation within this taxon has been associated with different ecoregions (Larson et al. 2004), suggesting that considerable variation may exist within this species for stress tolerance (Jones 2003). Currently, two cultivars, ‘Whitmar’ (released in 1946) and ‘Goldar’ (1989), and two recently released pre-variety germplasms, P-7 (2001) and Anatone (2003), are widely commercially available for restoration purposes.

Elymus wawawaiensis J. Carlson & Barkworth (Snake River wheatgrass) is another native, C₃, perennial Triticeae bunchgrass that is commonly utilized as a surrogate for *P. spicata*. This grass is allotetraploid ($2n = 4x = 28$) and is native to the drier regions of eastern Washington, eastern Oregon, and central and northern Idaho. While *P. spicata* may be awned or awnless, *E. wawawaiensis* is always awned. It can be distinguished from *P. spicata* by its greater spikelet overlap, more lanceolate glume shape, and greater degree of seedling pubescence (Jones et al. 1991; Carlson and Barkworth 1997). ‘Secar’ (1980) has been widely planted due to its superior establishment and drought tolerance compared to Whitmar and Goldar *P. spicata*. Due to taxonomic confusion, Secar was considered to be *P. spicata* until 1986 (Carlson and Barkworth 1997). In 2007, ‘Discovery’ was released as an intended alternative to Secar for its better establishment and drought tolerance (Jones 2008).

The importance of plant functional traits for restoration success has aroused considerable interest among restoration ecologists in the last few decades (Loreau et al. 2001; Naeem 2006). A better understanding and incorporation of species-specific functional traits may lead to successful prediction of a community’s response to environmental change (Suding and Goldstein 2008). For example, tolerance to drought or defoliation could be an important translator of organismic response to demographic response (Suding et al. 2003), which may vary across landscapes depending on the extremes of stress involved. In this research, I used plant functional traits as a tool to compare and evaluate different experimental and commercially available plant materials of the two native wheatgrasses, *P. spicata* and *E. wawawaiensis*. To develop an understanding of genetic variation for their morphological and physiological traits of

these species, I compared them for seedling traits, growth characteristics, and traits related to drought and defoliation tolerance. I intended to identify possible reasons for restoration failures involving these two native grass species and to suggest plant traits that could be used as indicators for better seedling establishment and superior drought and defoliation tolerance.

My three primary research goals were to evaluate native plant materials of *P. spicata* and *E. wawawaiensis* for:

1. germination success and seedling morphological and growth traits,
2. morphological and physiological traits for superior drought tolerance, and
3. morphological traits for superior defoliation tolerance.

I addressed the first objective (Chapters 2 and 3) by comparing Goldar, Whitmar, Anatone, six experimental populations of *P. spicata*, Secar *E. wawawaiensis* (Table 1), and the invasive annual *B. tectorum* in a 23-day growth-chamber study. I compared germination and seedling morphological traits (shoot and root biomass, shoot and root length, specific leaf area, and specific root length) and growth characteristics (absolute and relative growth rates) of these species and populations at low- and high-temperature regimes.

The second objective (Chapter 4), to evaluate drought tolerance capability, was dealt with in a greenhouse study. I compared Goldar, Anatone, two experimental populations of *P. spicata*, Secar, and one experimental population of *E. wawawaiensis* (Table 1) for their shoot and root biomass, root-to-shoot biomass ratio, specific leaf area, stomatal conductance, and water use efficiency at low, medium, and high watering frequencies.

The third objective (Chapters 5 and 6), to evaluate clipping tolerance, was accomplished in two field studies:

- defoliating Goldar, Anatone, three experimental populations of *P. spicata*, Secar, Discovery, and one experimental population of *E. wawawaiensis* (Table 1) repeatedly for two consecutive years and comparing them for shoot biomass, tiller number, leaf length, specific leaf area, basal area, and defoliation tolerance, and
- defoliating Whitmar, Goldar, Anatone, and nine experimental *P. spicata* populations once in spring for two consecutive years at two plant densities and comparing them for shoot and root biomass and defoliation tolerance.

References

- ADLER, P.B., D.G. MILCHUNAS, O.E. SALA, I.C. BURKE, AND W.K. LAUENROTH. 2005. Plant traits and ecosystem grazing effects: comparison of U.S. sagebrush steppe and Patagonian steppe. *Ecological Applications* 15: 774–792.
- ASAY, K.H., W.H. HORTON, K.B. JENSEN, AND A.J. PALAZZO. 2001. Merits of native and introduced Triticeae grasses on semiarid rangelands. *Canadian Journal of Plant Science* 81: 45-52.
- BOOTH, M.S., M.M. CALDWELL, AND J.M. STARK. 2003. Overlapping resource use in three Great Basin species: implications for community invisibility and vegetation dynamics. *Journal of Ecology* 91: 36-48.
- CALDWELL, M.M., J.H. RICHARDS, D.A. JOHNSON, R.S. NOWAK, AND R.S. DZUREC. 1981. Coping with herbivory: Photosynthetic capacity and resource allocation in two semi-arid *Agropyron* bunchgrasses. *Oecologia* 50: 14–24.

- CARLSON, J.R., AND M. BARKWORTH. 1997. *Elymus wawawaiensis*: a species hitherto confused with *Pseudoroegneria spicata* (Triticeae, Poaceae). *Phytologia* 83:312-330.
- HARRIS, G.A. 1967. Some competitive relationships between *Agropyron spicatum* and *Bromus tectorum*. *Ecological Monographs* 37:89-111.
- HARRIS, G.A., AND A.M. WILSON. 1970. Competition for moisture among seedlings of annual and perennial grasses as influenced by root elongation at low temperature. *Ecology* 51: 530–534.
- JENSEN, K.B., M. CURTO, AND K.H. ASAY 1995. Cytogenetics of Eurasian bluebunch wheatgrass and their relationship to North American bluebunch and thickspike wheatgrass. *Crop Science* 35: 1157-1162.
- JONES, T.A. 2003. The Restoration Gene Pool concept: beyond the native versus non-native debate. *Restoration Ecology* 11: 281-290.
- JONES, T.A. 2008. Notice of release of ‘Discovery’ Snake River wheatgrass. *Native Plants Journal* 9: 99-101.
- JONES, T.A., D.C. NIELSON, AND J.R. CARLSON. 1991. Development of a grazing-tolerant native grass for revegetating bluebunch wheatgrass sites. *Rangelands* 13:147-150.
- LARSON, S.R., T.A. JONES, AND K.B. JENSEN. 2004. Population structure in *Pseudoroegneria spicata* (Poaceae: Triticeae) modeled by Bayesian clustering of AFLP genotypes. *American Journal of Botany* 11:1788-1800.
- LOREAU, M., S. NAEEM, P. INCHAUSTI, J. BENGTSSON, J.P. GRIME, A. HECTOR, D.U. HOOPER, M.A. HUSTON, D. RAFFAELLI, B. SCHMID, D. TILMAN, AND D.A.

- WARDLE. 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* 294: 804-808.
- MACK, R.N., AND J.N. THOMPSON. 1982. Evolution in steppe with few large, hooved mammals. *The American Naturalist* 119: 757-773.
- MILLER, R.F., J.M. SEUFERT, AND M.R. HAFERKAMP. 1986. The ecology and management of bluebunch wheatgrass (*Agropyron spicatum*): a review. *Oregon Agricultural Experiment Station Bulletin*. 669.
- MONSEN, S. B., R. STEVENS, AND N. L. SHAW. 2004. Restoring western ranges and wildlands (Vol. 2). Fort Collins, CO, USA. U.S. Forest Service Gen. Tech. Rep. RMRS-GTR 136. 698 p.
- NAEEM, S. 2006. Biodiversity and ecosystem functioning in restored ecosystems: extracting principles for a synthetic perspective. In: Falk, D.A., M. A. Palmer, and J. B. Zelder (EDS) *Foundation of restoration ecology*. Island Press, Washington. p 210-237.
- RICHARDS, R.T., J.C. CHAMBERS, AND C. ROSS. 1998. Use of native plants on federal lands: policy and practice. *Journal of Range Management* 51: 621-632.
- SUDING, K.N., AND L.J. GOLDSTEIN. 2008. Testing the Holy Grail framework: using functional traits to predict ecosystem change. *New Phytologist* 180: 559-562.
- SUDING, K.N., D.E. GOLDBERG, AND K.M. HARTMAN. 2003. Relationships among species traits: separating levels of response and identifying linkages to abundance. *Ecology* 84: 1-16.
- WHISENANT, S.G. 1999. Repairing damaged wildlands: a process-oriented, landscape-scale approach. Cambridge, UK: Cambridge University Press. 312 p.

YOUNG, J.A., AND C.D. CLEMENTS. 2007. Cheatgrass and grazing rangelands. *Rangelands* 26: 15-20.

YOUNG, J.A., AND F.L. ALLEN. 1997. Cheatgrass and range science: 1930-50. *Journal of Range Management* 50: 530–535.

Table 1. Cultivars (C), germplasms (G), and experimental populations of *P. spicata* and *E. wawawaiensis* and their source of origin.

Populations	Source of origin
<i>Pseudoroegneria spicata</i>	
P-1 *	Several unknown locations
P-3 *	Grande Ronde River, Oregon
P-7 (C) *	25 locations - multiple origin polycross
P-9 _t	Colchicine doubled from P-1
P-22	Selected from P -1 (for clipping tolerance)
P-24	Selected from Acc: 238
P-26	from 25 locations - developed plant material (from P-7)
P-27 _t	Colchicine doubled from P-3
Acc: 238 *	Lind, Washington
Whitmar (C) *	Colton, Washington
Goldar (C) *	Umatilla National Forest, Washington
Anatone (G) *	Anatone, Washington
<i>Elymus wawawaiensis</i>	
Secar (C) *	Lewiston, Idaho
Discovery (G) *	From 10 location, north-eastern Washington, central Idaho
E-46	Developed from Discovery for clipping tolerance

‘t’ indicates tetraploid *P. spicata* populations

CHAPTER 2

IMMATURE SEEDLING GROWTH OF TWO NORTH AMERICAN NATIVE
PERENNIAL BUNCHGRASSES AND THE INVASIVE GRASS
*BROMUS TECTORUM*¹

Abstract

The success of *Bromus tectorum* L., an invasive winter annual grass of North America's Intermountain West, has been attributed to its early germination, superior cold-temperature growth, profuse root production, and high specific leaf area (SLA). We compared *B. tectorum* to commercially available plant materials of two perennial rangeland bunchgrasses native to the region, *Pseudoroegneria spicata* (Pursh) A. Löve (cv. Whitmar, cv. Goldar, and Anatone Germplasm) and *Elymus wawawaiensis* J. Carlson & Barkworth (cv. Secar) to identify perennial-grass traits that likely contribute to successful seedling establishment. Specifically, we measured seed germination, seedling morphological traits, and growth rates at the immature seedling stage. The experiment was conducted under cold (5/10°C) and more favorable (15/20°C) day/night temperatures, resembling typical early spring and late spring temperatures, respectively, of the Intermountain West. Our data suggest that, at the early seedling stage, a substantial investment in root surface area, rather than high shoot length or SLA, accounts for *B. tectorum*'s success. *Pseudoroegneria spicata*, in particular Anatone Germplasm, exhibited highest shoot biomass, root biomass, shoot length, and absolute growth rate. However, *E. wawawaiensis* displayed highest SLA at both temperatures and highest specific root length (SRL) at low temperature, despite displaying the lowest shoot and root biomass. Although *P. spicata*'s greater productivity may initially make for better

¹ Coauthored by: Jayanti Ray Mukherjee, TA Jones, PB Adler, TA Monaco

seedling establishment than *E. wawawaiensis*, it may also prove disadvantageous in competitive or highly resource-limited environments where high SRL could be an advantage.

Introduction

Past grazing practices, invasion by flammable weeds, and resultant increased fire frequency have contributed to declines in the abundance of perennial bunchgrasses, such as *Pseudoroegneria spicata* (Pursh.) A. Löve, on the rangelands of North America's Intermountain West (Daubenmire 1942; Young and Allen 1997; Whisenant 1999). Consequently, this C₃ species is widely used for restoration applications in the region (Monsen et al. 2004). Prior to 1986, another C₃ perennial Triticeae bunchgrass, *E. wawawaiensis* J. Carlson & Barkworth, was taxonomically confused with *P. spicata*, owing to their superficial morphological resemblance (Carlson and Barkworth 1997). Since the commercial release of 'Secar' *E. wawawaiensis* in 1980, this cultivar has become widely and successfully used as a restoration surrogate for *P. spicata*. However, the realization that Secar is not *P. spicata* has made its use somewhat controversial. In addition, the natural distribution of *E. wawawaiensis* is mostly limited to eastern Oregon, eastern Washington, and northern and central Idaho (Morrison and Kelley 1981; Carlson and Barkworth 1997), while most restoration sites where *E. wawawaiensis* is used fall outside of this area. Nevertheless, Secar *E. wawawaiensis* continues to be more widely used than any *P. spicata* plant material in the Intermountain West (Young and Allen 1997; Monsen et al. 2004; Lambert 2006).

When present, invasive annual grasses, particularly *Bromus tectorum* L., may limit native grass seedling establishment (Harris and Wilson 1970; Young and Allen

1997) and replace native vegetation (Sperry et al. 2006). Invasion by *B. tectorum* greatly increases fire frequency (Brooks et al. 2004), consequently damaging native vegetation (Whisenant 1990) and possibly modifying the soil environment in a way that reinforces its dominance (Saetre and Stark 2005; Norton et al. 2007).

The success of *B. tectorum* has been attributed to superior germination, growth, and fitness traits. For example, it has the ability to germinate in fall, giving it the ability to put spring-germinating perennial seedlings at a competitive disadvantage (Hardegree and Van Vactor 2004); to germinate earlier in the spring than perennial grasses (Harris 1967; Harris and Wilson 1970; Harris 1977; Hardegree and Van Vactor 2004); to grow vigorously above (Svejcar 1990) and below ground (Harris and Wilson 1970; Harris 1977; Young and Allen 1997; Arredondo et al. 1998) when temperatures are cold and soil moisture is least limiting (Bradford and Lauenroth 2006); and to produce large quantities of seeds in summer that give it a numerical advantage (Svejcar 1990; Young and Allen 1997; Sperry et al. 2006).

Additionally, *B. tectorum*'s success has been attributed to functional traits such as high relative growth rate (RGR) (Arredondo et al. 1998), high specific leaf area (SLA) (Svejcar 1990), and high specific root length (SRL) (Svejcar 1990). The high RGR of invasive species is thought to be a major factor contributing to their invasive ability (Grotkopp et al. 2002; James and Drenovsky 2007), and annuals are known to have higher RGR than perennials (Poorter and Garnier 2007). Specific leaf area is generally considered to be the best predictor for RGR (Poorter and Garnier 2007), and high RGR often reflects high resource-uptake rates exhibited by invasive species (Grotkopp et al. 2002). Species with high SRL are known for rapid extraction of water and nutrients

(Eissenstat 1991; Ryser 2006), and high SRL is often associated with high SLA and RGR (Poorter and Garnier 2007). High SRL of *B. tectorum* at the immature seedling stage enables acquisition and utilization of early-season resources that typically diminish over the course of the season (Svejcar 1990; Ryser 2006). However, most of these studies compare established seedlings or mature plants, and functional-trait expression of germinating seedlings remains mostly unexplored (Jones et al. 2010).

A better understanding of growth and development of germinating perennial grass seedlings may lead to development of improved plant materials with more successful establishment, particularly under cold-temperature conditions. To achieve this end, it is important to identify the root traits that contribute to establishment success (Svejcar 1990) and native plant materials that possess these traits (Jones et al. 2010). In this study, our objectives were: 1) to identify immature seedling traits that may contribute to *B. tectorum*'s rapid growth rate and success relative to the two perennial grasses (*P. spicata*, *E. wawawaiensis*) native to the sagebrush-steppe rangelands of the Intermountain West; 2) to determine which commercially available native plant materials of these two native bunchgrasses display the best immature seedling-growth characteristics; and 3) to reveal traits associated with perennial-grass seedling productivity that are likely predictors of superior growth rate. To this end, we compared three commercially available *P. spicata* populations ['Whitmar' (released in 1946), 'Goldar' (1989), and Anatone Germplasm (2003)], 'Secar' (1980) *E. wawawaiensis*, and *B. tectorum* for germination, seedling morphological traits, and seedling growth under favorable and unfavorable (cold) temperature conditions. In addition to days to germination, days to shoot initiation, shoot and root biomass, absolute growth rate (AGR), and RGR, we examined seedling

morphological growth traits such as shoot and root length, SLA, SRL, root-to-shoot (R:S) biomass ratio, and R: S length ratio.

We predicted that *B. tectorum* would germinate sooner, produce greater shoot and root biomass, produce greater shoot and root length, and display higher R: S biomass and R: S length ratios and greater SLA, and SRL than *P. spicata* and *E. wawawaiensis*, particularly under low temperature. We also predicted that *B. tectorum* would display the greatest seedling RGR and that perennial plant materials with levels of trait expression most similar to *B. tectorum* would also display the greatest seedling RGR. In addition, we wished to compare *P. spicata* and *E. wawawaiensis* for seedling shoot and root biomass under the two temperature regimes. Based on previous results (Gibbs et al. 1991; Kitchen and Monsen 1994), we anticipated that the more recently released plant materials of *P. spicata*, Goldar and particularly Anatone Germplasm, would display superior seedling growth characteristics relative to Whitmar, the first released *P. spicata* plant material.

Materials and methods

We employed three released populations of *P. spicata* (Goldar, Whitmar, and Anatone Germplasm), Secar *E. wawawaiensis*, and *B. tectorum* in this study. Seeds of all four perennial populations were produced in a common environment at Utah State University's Evans Farm at Millville, UT to avoid bias from confounded maternal effects resulting from multiple seed-production environments. While all seeds were produced in the same field, individual seed-production plots were established perpendicular to the prevailing wind and isolated by distance to minimize cross-pollination between plots. Seeds were harvested in 2007, cleaned, and refrigerated (5°C) until experimental use. *Bromus tectorum* seeds were harvested in 2005 from multiple locations in Idaho, USA,

bulked, cleaned and refrigerated until experimental use. Simultaneously, we conducted a laboratory germination trial with seeds collected from these sources with approximately 90 to 94% germination success of all seed sources.

Two growth chambers (Percival Scientific, Inc., Perry, IA, USA) were randomly assigned to high and low temperature regimes. Actual temperatures [alternating 12-hour 20 ± 1.25 °C days/ 15 ± 2.16 °C nights (high) and 10 ± 0.33 °C days/ 5 ± 0.28 °C nights (low)] were recorded using a data logger (Watchdog, Spectrum Technologies, Plainfield, IL, USA) in each chamber. Light intensity was calculated (average 191.4 ± 9 $\mu\text{moles m}^{-2} \text{sec}^{-1}$ across temperatures) using a LI COR Model LI-185B photometer with a LI-190SB quantum sensor (LI-COR Biosciences, Lincoln, Nebraska). The two temperature regimes were intended to correspond to typical early- and late-spring temperatures of the North American Intermountain West (Limbach and Call 1996).

A total of 1,000 seeds across two temperature regimes and three species, including the three *P. spicata* populations, were arranged in a completely randomized design. Four seeds (subsamples) were placed in a single germination pouch (CYG seed germination pouch, Mega International, St. Paul, MN, USA), which is a folded plastic sheet enclosing a blotter paper. Twenty-five pouches (replicates) of each population were used in each growth chamber. Thus, a total of 100 seeds of each population were evaluated for each temperature.

We sprayed blotter papers to saturation with same amounts of water with spray bottles filled with distilled water, and pouches were rewatered as necessary throughout the experiment to maintain desirable moisture conditions. The study was initiated on 12 Aug. 2008, each seed was monitored daily, and the dates of first appearance of radicle

(approximately 1mm) and coleoptile (1 mm) were recorded as germination and shoot initiation. Germination pouches were randomly rotated each week within the growth chambers.

Seedlings from 12 replicates were destructively harvested eight days following initial watering for the high-temperature treatment and 16 days for the low-temperature treatment. The remaining 13 replicates were harvested 7 days following the first harvest for both temperatures. The low-temperature harvest was delayed relative to high temperature to allow seedlings to be sufficiently sizeable for measuring seedling traits. At harvest, a digital image of each seedling was obtained with a flatbed scanner (300 dpi). Images were analyzed with WinRHIZO Pro Version 2005b (Reagent Instrument Inc., Québec City, Canada) to determine leaf area, leaf length, and root length. Subsequently, shoots and roots were separated and oven dried (60°C for 48 hours) to determine dry mass. Scanned images were analyzed for shoot and root length, leaf area, SLA ($\text{cm}^2 \text{mg}^{-1}$), and SRL (cm mg^{-1}). Absolute growth rate (AGR) was calculated as $\text{AGR} = [\mu(W_2) - \mu(W_1)] / (t_2 - t_1)$, and relative growth rate was calculated as $\text{RGR} = [\mu(\ln W_2) - \mu(\ln W_1)] / (t_2 - t_1)$, where W_1 and W_2 were the dry masses (shoot + root) from the first and second harvests, respectively, and μ was the mean biomass for the indicated harvest.

Data were analyzed using SAS 9.1.3 (SAS Institute 2003) and transformed as necessary to meet normality assumptions. Means were back-transformed for presentation. In addition, heterogeneous variances were corrected using a REPEATED (Littell et al. 1996) statement in PROC MIXED. This reduced within-temperature heterogeneity by partitioning the residual variance into separate groups for each temperature. For mean comparisons, one-way analysis of variance was performed in PROC MIXED, and the

contrasts and their interactions were generated in PROC GLM. We used data from both harvests to calculate days to germination, days to shoot initiation, AGR, and RGR, while the remaining traits were determined for the second harvest alone. Because days to harvest differed between temperature regimes and because we had no replication of growth chambers, we did not directly compare high- and low-temperature treatments with a temperature main effect for seedling traits or growth rates, as this would be statistically invalid. However, we did assess the species x temperature and *P. spicata* populations x temperature interactions for these variables. Absolute growth rate and RGR were calculated separately for high and low temperatures and a significant ($P < 0.05$) interaction between harvest and species (or *P. spicata* populations) for total biomass was interpreted as a significant difference between species (or *P. spicata* populations) for growth rate. Likewise, the same interactions for $\ln(\text{total biomass})$ were used to test for differences among species and populations for RGR. Differences between species and populations were calculated using ESTIMATE statements in SAS.

Results

Bromus tectorum germinated (initiated roots) approximately two days earlier than *P. spicata* and Secar *E. wawawaiensis* at high temperature (Table 1, Fig. 1a), and this difference was approximately three days at low temperature. The two native perennial species did not differ for days to germination, and their response was similar under both temperatures. *Pseudoroegneria spicata* populations did not differ for days to germination, but they did interact with temperature (Table 1). At low temperature, the three *P. spicata* populations were similar in days to germination, while at high temperature, days to germination for Whitmar was faster than for Anatone, with Goldar being intermediate.

Bromus tectorum initiated shoots (coleoptiles) two days earlier than *P. spicata* and Secar *E. wawawaiensis* at low temperature and one day earlier at high temperature (Table 1, Fig. 1b). Across temperatures, *P. spicata* initiated shoots slightly earlier (0.2 d) than *E. wawawaiensis*. Among the three *P. spicata* populations, Whitmar and Anatone initiated shoots earlier than Goldar at low temperature, while at high temperature the populations were similar.

On average, *P. spicata* produced 19% greater shoot biomass than *B. tectorum*, while *B. tectorum* produced 29% greater shoot biomass than Secar *E. wawawaiensis* (Table 1, Fig. 1c). Although *P. spicata* produced greater shoot biomass than Secar *E. wawawaiensis* under both temperatures, this difference was greater at high temperature. *Pseudoroegneria spicata* populations interacted with temperature for shoot biomass. All three populations produced similar shoot biomass at low temperature, while Anatone and Goldar produced greater shoot biomass than Whitmar at high temperature.

Across temperatures, root biomass of *P. spicata* and *B. tectorum* was similar, but root biomass of Secar *E. wawawaiensis* was lower (Table 1, Fig. 2a). However, the two native perennials interacted with temperature for root biomass. At low temperature, *P. spicata* produced about 88% greater root biomass than *E. wawawaiensis*, while at high temperature its root biomass was only 41% greater. *Pseudoroegneria spicata* populations neither differed nor interacted with temperature for this trait (Table 1).

Bromus tectorum's R:S biomass ratio exceeded that of the perennials (Table 1, Fig. 1d). The perennial species did not differ for this trait, but did interact with temperature. The *P. spicata* populations produced an average of 29% higher R:S biomass ratio than Secar *E. wawawaiensis* at low temperature, while Secar's R:S biomass ratio was

15% higher at high temperature. Populations within *P. spicata* differed for R:S biomass ratio similarly across temperatures, with Anatone and Whitmar being similar and both being greater than Goldar (Fig. 1d).

Among species, *P. spicata* was greatest for shoot length at both temperatures, followed by Secar *E. wawawaiensis*, and finally *B. tectorum* (Table 1, Fig. 1e). However, these differences were greater under the high-temperature regime. Although all *P. spicata* populations exhibited similar shoot length at low temperature, Anatone had the greatest shoot length, followed by Goldar, and then by Whitmar at high temperature (Fig. 1e).

Bromus tectorum and the perennials differed for root length and interacted with temperature for this trait (Table 1, Fig. 1f). Under both temperature regimes, *B. tectorum* produced greater root length than *P. spicata*, while Secar *E. wawawaiensis* was lowest. However, the difference between *B. tectorum* and the perennials was heightened at low temperature. The *P. spicata* populations also differed for root length, with Anatone being highest and Goldar being lowest, but they did not interact with temperature.

All three species differed for R:S length ratio and also interacted with temperature for this trait. At low temperature, R:S length ratio of *Bromus tectorum* was 63% greater than *P. spicata* and 87% greater than *E. wawawaiensis*, while at high temperature *B. tectorum*'s R:S length ratio was approximately 94% greater than both the native species (Table 1, Fig. 1g). *Pseudoroegneria spicata* populations were similar for R:S length ratio at low temperature, while at high temperature, Goldar's R:S length ratio was significantly less than that of Whitmar, with Anatone being intermediate.

All three species differed in SLA and also interacted with temperature for this trait (Table 1, Fig. 2b). The SLA of Secar *E. wawawaiensis* was highest, followed by *P.*

spicata, and finally *B. tectorum*, but the superiority of Secar was greater at high temperature. No differences for SLA were seen among *P. spicata* populations. Overall, the perennials did not differ from *B. tectorum* for SRL, but they did they vary from each other and interacted with temperature (Table 1, Fig. 2c). At low temperature, Secar *E. wawawaiensis* produced approximately 50% greater SRL than *P. spicata*, while at high temperature, the two species were similar. Like SLA, SRL neither differed among *P. spicata* populations nor did they vary with temperature.

A significant interaction between harvest and species indicated that species ($P < 0.0001$) were significantly different for AGR, but a non-significant interaction ($P > 0.05$) between harvest and *P. spicata* populations indicated that AGR of these populations was similar (Table 1, Fig. 3a). However, we found a significant ($P < 0.05$) three-way interaction between temperature, harvest, and *P. spicata* populations, indicating that these populations differed for AGR at high temperature, but not at low temperature. On average, *P. spicata* displayed highest AGR under both temperature regimes, while *B. tectorum* exhibited the least AGR. Within *P. spicata*, populations were similar for AGR at low temperature, while at high temperature Anatone and Goldar were greater than Whitmar. Species differed ($P < 0.0001$) for RGR (ln-transformed total biomass), but *P. spicata* populations were similar ($P > 0.05$) under both temperature regimes (Table 1, Fig. 3b). On average, RGR was highest for *P. spicata*, followed by Secar *E. wawawaiensis*, and then *B. tectorum* (Fig. 3b).

Discussion

Our first objective was to identify additional seedling traits that may contribute to *B. tectorum*'s success. Overall, the most striking difference displayed by *B. tectorum*

compared to the native perennials was the former's exceptionally high R:S length ratio, which was evident at both temperatures. In addition, in accordance with our prediction, *B. tectorum* produced greater R:S biomass ratio under both temperature regimes. These data suggest a *B. tectorum* strategy of emphasizing root biomass, and particularly root surface area, in young seedlings at the expense of photosynthetic biomass and area. In semi-arid environments, this strategy may pay dividends as the season progresses and plant competition increases for declining soil moisture.

Our second objective was to compare the four commercially available perennial populations for immature seedling characteristics that may contribute to enhanced seedling growth. We found that *P. spicata* produced greater shoot biomass, root biomass, and shoot length under both temperature regimes. Low temperature affected shoot biomass more for *P. spicata* than for Secar *E. wawawaiensis*, while the opposite trend was observed for root biomass. Thus, low temperature reduced R:S biomass ratio 28% for Secar, but only 6% for *P. spicata*.

Consistent with our prediction, *B. tectorum* germinated and initiated shoots earlier than the perennials under both temperature regimes, and this difference was greater at cold temperature. Mean germination time is considered to be a highly phylogenetically conserved trait (Norden et al. 2009), and early germination may be associated with a species' invasive ability (Grotkopp et al. 2002). However, early germination of *B. tectorum* was not associated with high AGR or RGR at the early seedling stage, contrary to our expectation (Aguirre and Johnson 1991; Grotkopp et al. 2002). While the disparity in AGR may be attributed to the small seed mass ($2.63 \text{ mg seed}^{-1}$) of the annual compared to the perennials ($3.76 \text{ mg seed}^{-1}$), a substantially lower RGR of *B. tectorum*

than that of both perennials was associated with low SLA. Relative growth rate may change over time in a plant's life cycle (Hunt 1982), and typically RGR is highest (RGR_{max}) during the first few days post-germination and then declines over time. A low RGR for *B. tectorum* could thus be a consequence of the growth phase at which measurements are taken, and differences in RGR might be a result of species differences in timing of RGR_{max} (Hunt 1982; Grotkopp et al. 2002). Because *B. tectorum* germinates in the fall, it may require seedling tissues that are well constructed (low SLA and SRL) to overwinter. At the same time, it is conceivable that, once spring arrives, *B. tectorum* shifts its physiological strategy in favor of greater surface area to fuel faster growth.

While *P. spicata* displayed higher AGR, which we attribute to its larger seed mass ($3.76 \text{ mg seed}^{-1}$) relative to *E. wawawaiensis* ($2.88 \text{ mg seed}^{-1}$), the two perennial species were similar for RGR. As predicted, SLA for *E. wawawaiensis* was consistently greater than for *P. spicata* at both temperatures. In addition, Secar had 50% greater SRL than *P. spicata* at low temperature. As shown in previous studies, high SLA, SRL, and RGR are properties of fast-growing species (Poorter and Garnier 2007). Consequently, Secar may be well equipped by its high SLA and SRL to compensate for its low initial biomass as the growing season progresses (Zhang and Muan 1990).

The three *P. spicata* populations were similar for most of the traits with five exceptions: Goldar initiated shoots one day later than Anatone and Whitmar at low temperature, Whitmar shoot biomass was lowest at high temperature, Anatone's shoot length was greatest followed by Goldar and then Whitmar at high temperature, Anatone's root length was greater than Goldar's with Whitmar intermediate for root biomass at high temperature, and Whitmar had lower AGR and RGR than Anatone and Goldar at high

temperature. For high temperature, these results are consistent with our expectation that the more recently released populations, Anatone and Goldar, would be superior to Whitmar, but no such trend was seen for low temperature.

Our final objective was to identify traits associated with superior seedling growth. Our prediction that perennials most similar to *B. tectorum* would display the greatest RGR was based on the assumption that *B. tectorum* would feature the greatest RGR. However, at the immature seedling stage, this assumption turned out to be false. Nevertheless, we were able to identify traits in the perennial species that are likely to contribute to superior seedling growth rate. For AGR, *P. spicata* was clearly superior to *E. wawawaiensis*, while the two species were similar for RGR. Traits associated with high AGR in *P. spicata* were high length and biomass of shoots and roots, while traits that likely contributed to high RGR in *E. wawawaiensis* were high SLA and SRL. Specific leaf area and SRL are two traits closely associated with plant life-history, and species with high SLA and SRL are associated with high competitive and invasive ability (Grotkopp et al. 2002) and resource-rich environments (Reich et al. 1998; Ryser 2006). Nevertheless, despite originating from a more arid locale than any of the three *P. spicata* populations, Secar *E. wawawaiensis* displayed very high SLA and SRL (Monsen et al. 2004). We believe that high SLA and SRL are two traits that may be crucial for future selection of perennial plant materials for restoration. High SLA may confer high growth rate, and high SRL may enable greater resource extraction (Svejcar 1990; Arredondo et al. 1998).

In conclusion, our comparison at the immature seedling stage reveals two traits that contribute to *B. tectorum*'s successful establishment: 1) rapid germination and 2) a

high investment in root length relative to shoot length, rather than a high investment in root biomass relative to shoot biomass. While we predicted that perennial species that displayed trait levels most similar to *B. tectorum* would display greatest seedling growth, we found that *P. spicata* and *E. wawawaiensis* were quite similar for the two above-mentioned traits, particularly when compared to *B. tectorum*. In spite of this, inspection of other traits revealed fundamental differences that elucidate contrasting seedling-growth strategies for these two perennial grasses. While *P. spicata*'s high AGR provides an advantage for initial seedling establishment, its relatively low SLA and its greater investments in biomass may eventually limit its growth rate (Harris 1977; Reich et al. 1998), when resources are diminishing. Secar *E. wawawaiensis*, on the other hand, displays greater above- and below-ground surface area per unit biomass that likely contributes to biomass accumulation and competitive ability once established.

Implications

Bromus tectorum is an extremely effective competitor with perennial grasses, particularly under cold temperatures. The ability of perennial grass seedlings to effectively compete with fall-germinating *B. tectorum* is unlikely, but establishment of seeded perennial grasses could be improved if seedlings displayed improved cold-temperature growth in spring, making them more competitive with spring-germinating *B. tectorum*. Some have suggested that perennials that possess traits common to invasive annuals will be more competitive. However, because annual and perennial grasses have many fundamental differences, developing perennial plant materials that are functionally similar to invasive annuals would be a tremendous challenge. Furthermore, while the two perennial grasses studied here may not be the most effective competitors due to their mid-

to-late seral status, results of this research provide clues for selecting for improved cold-temperature growth potential in early-seral perennial grass species such as sandberg bluegrass (*Poa secunda* J. Presl), big squirreltail (*Elymus multisetus* [J.G. Sm.] Burt Davy), and bottlebrush squirreltail (*E. elymoides* [Raf.] Swezey).

References

- AGUIRRE, L., AND D. A. JOHNSON. 1991. Root morphological development in relation to shoot growth in seedlings of four range grasses. *Journal of Range Management* 44: 341–346.
- ARREDONDO, J. T., T. A. JONES, AND D. A. JOHNSON. 1998. Seedling growth of Intermountain perennial and woody annual grasses. *Journal of Rangeland Management* 54: 584–589.
- BRADFORD, J. B., AND W. K. LAUENROTH. 2006. Controls over invasion of *Bromus tectorum*: the importance of climate, disturbance, soil and seed availability. *Journal of Vegetation Science* 17: 693–704.
- BROOKS, M. L., AND C. M. D'ANTONIO, D. M. RICHARDSON, J. B. GRACE, J. E. KEELEY, J. M. DiTOMASO, R. J. HOBBS, M. PELLANT, AND D. PYKE. 2004. Effect of invasive alien plants on fire regimes. *Bio Science* 54: 677–688.
- CARLSON, J. R., AND M. E., BARKWORTH. 1997. *Elymus wawawaiensis*: a species hitherto confused with *Pseudoroegneria spicata* (Triticeae, Poaceae). *Phytologia* 83: 312–330.
- DAUBENMIRE, R. F. 1942. An ecological study of the vegetation of southeastern Washington and adjacent Idaho. *Ecological Monographs* 12: 53–79.

- EISSENSTAT, D. M. 1991. On the relationship between specific root length and the rate of root proliferation: a field study using citrus rootstocks. *New Phytologist* 118: 63-68.
- GIBBS, J. L., G. YOUNG, AND J. R. CARLSON. 1991. Registration of 'Goldar' bluebunch wheatgrass. *Crop Science* 31: 1708.
- GROTKOPP, E., M. REJMÁNEK, AND T. L. ROST. 2002. Toward a causal explanation of plant invasiveness: seedling growth and life history strategies of 29 pine (*Pinus*) species. *American Naturalist* 159: 396–419.
- HARDEGREE, S. P., AND S. S. VAN VACTOR. 2004. Microclimatic constraints and revegetation planning in a variable environment. *Weed Technology* 18: 1213-1215.
- HARRIS, G. A. 1967. Some competitive relationships between *Agropyron spicatum* and *Bromus tectorum*. *Ecological Monographs* 37: 89-111
- HARRIS, G. A. 1977. Root phenology as a factor of competition among grass seedlings. *Journal of Range Management* 30: 172–177.
- HARRIS, G. A., AND A. M. WILSON. 1970. Competition for moisture among seedlings of annual and perennial grasses as influenced by root elongation at low temperature. *Ecology* 51: 530–534.
- HUNT, R. 1982. Plant growth curves: the functional approach to plant growth analysis. London, United Kingdom: Edward Arnold Ltd. 248p.
- JAMES, J. J., AND R. E. DRENOVSKY. 2007. A basis of relative growth rate differences between native and invasive forb seedlings. *Rangeland Ecology and Management* 60: 395–400.

- JONES, T. A., T. A. MONACO, AND J. J. JAMES. 2010. Launching the counterattack: Interdisciplinary deployment of functional traits to repair Intermountain rangelands damaged by annual grass invasion. *Rangelands* 32: 38-42.
- KITCHEN, S. G., AND S. B. MONSEN. 1994. Germination rate and emergence success in bluebunch wheatgrass. *Journal of Range Management* 47: 145-150.
- LAMBERT, S. 2006. Seed use by Bureau of Land Management. Available at: <http://www.nps.gov/plants/meetings/mohave2006/Lambert-Mohave2006.pdf>. Accessed on 18 Oct 2009.
- LIMBACH, W.C., AND CALL, C.A. 1996. Germination response of Russian wildrye to variations in seed mass at fluctuating temperatures. *Journal of Range Management* 49: 346-349.
- LITTELL, R. C., G. A. MILLEKEN, W. W. STROUP, AND R. D. WOLFINGER. 1996. SAS system for mixed models. Cary, NC, USA: SAS Institute Inc. 633 p.
- MONSEN, S. B., R. STEVENS, AND N. L. SHAW. 2004. Restoring western ranges and wildlands (Vol. 2). Fort Collins, CO, USA. U.S. Forest Service Gen. Tech. Rep. RMRS-GTR 136. 698 p.
- MORRISON, K. J., AND C. A. KELLEY. 1981. Secar bluebunch wheatgrass. EB 0991. Cooperative Extension, Pullman, WA, USA: Washington State University. 4 p.
- NORDEN, N., M. I. DAWS, C. ANTOINE, M. A. GONZALEZ, N. C. GARWOOD, AND J. CHAVE. 2009. The relationship between seed mass and mean time to germination for 1037 tree species across five tropical forests. *Functional Ecology* 23: 203–210.

- NORTON, J. B., T. A. MONACO, AND U. NORTON. 2007. Mediterranean annual grasses in Western North America: kids in a candy store. *Plant and Soil* 298: 1-5.
- POORTER, H., AND E. GARNIER. 2007. Ecological significance of inherent variation in relative growth rate and its components. In: Pugnaire FI, and F. Valladares (EDS.). *Functional Plant Ecology*. Boca Raton, FL, USA: CRC Press, pp 67–100.
- REICH, P. B., M. G. TJOELKER, M. B. WALTERS, D. W. VANDERKLEIN, AND C. BUSCHENA. 1998. Close association of RGR, leaf and root morphology, seed mass, and shade tolerance in seedlings of nice boreal tree species grown in high and low light. *Functional Ecology* 12: 327-338.
- RYSER, P. 2006. The mysterious root length. *Plant and Soil* 286: 1-6.
- SAETRE, P., AND J. M. STARK. 2005. Microbial dynamics and carbon and nitrogen cycling following re-wetting of soils beneath two semi-arid plant species. *Oecologia* 142: 247-260.
- SAS [COMPUTER PROGRAM]. 2003. SAS Version 9.1.2. Cary, NC, USA: SAS Institute, Inc.
- SPERRY, L. J., J. BELNAP, AND R. D. EVANS. 2006. *Bromus tectorum* invasion alters nitrogen dynamics in an undisturbed grassland ecosystem. *Ecology* 87: 603–615.
- SVEJCAR, T. J. 1990. Root length, leaf area, and biomass of crested wheatgrass and cheatgrass seedlings. *Journal of Range Management* 43: 446–448.
- WHISENANT, S. G. 1990. Changing fire frequencies on Idaho's Snake River Plains: ecological and management implications. p. 4-10. In: McArthur, E. D., E. M. Romney, S. D. Smith, and P T. Tueller. [EDS.]. *Proceedings – symposium on cheatgrass invasion, shrub die-off, and other aspects of shrub biology and*

management. 5-7 April 1989. Las Vegas, NV. Gen. Tech. Rep. INT-276. USDA Forest Service, Ogden, UT, USA. Intermountain Research Station.

WHISENANT, S. G. 1999. Repairing damaged wildlands: a process-oriented, landscape-scale approach. Cambridge, UK: Cambridge University Press. 312 p.

YOUNG, J. A., AND ALLEN, F. L. 1997. Cheatgrass and range science: 1930-50. *Journal of Range Management* 50: 530–535.

ZHANG, J., AND M. A. MUAN. 1990. Effects of sand burial on seed germination, seedling emergence, survival, and growth of *Agropyron psammophilum*. *Canadian Journal of Botany* 68: 304–310.

Table 1. *F*-values for (a) overall analysis of variance for 10 seedling traits of three populations of *P. spicata*, Secar *E. wawawaiensis*, and *B. tectorum* and (b) species and temperature x species contrasts for 10 seedling traits.

(a)

Traits	Species	Among <i>P. spicata</i> populations	Temperature* species	Temperature* <i>P. spicata</i> populations
<i>Numerator df</i>	2	2	2	2
Days to root initiation	232.21***	0.83	18.78***	3.41*
Days to shoot initiation	67.25***	20.08***	3.34*	12.52***
Shoot (S) biomass (mg)	38.32***	3.33*	5.64**	3.53*
Root (R) biomass (mg)	21.86***	1.58	0.81	1.05
R: S biomass ratio	6.42**	7.89**	9.90**	0.25
Shoot length (mm)	118.79***	8.02**	23.79***	6.35*
Root length (mm)	23.68***	4.75*	2.46	0.71
R: S length ratio	168.77***	2.26	6.06*	3.51*
SLA (cm ² mg ⁻¹)	39.41***	2.10	1.06	1.27
SRL (cm mg ⁻¹)	9.12**	0.33	4.56*	2.02

(b)

Traits	Annual vs. perennials	<i>P. spicata</i> vs. <i>E. wawawaiensis</i>	Temperature* Annual vs. perennials	Temperature* <i>P. spicata</i> vs. <i>E. wawawaiensis</i>
<i>Numerator df</i>	1	1	1	1
Days to root initiation	577.64***	0.51	47.87***	0.01
Days to shoot initiation	186.79***	8.02**	9.79**	0.37
Shoot (S) biomass (mg)	4.51*	103.22***	3.48	11.88**
Root (R) biomass (mg)	1.00	66.37***	0.12	2.22**
R: S biomass ratio	15.27***	0.49	1.71	19.64**
Shoot length (mm)	196.84***	82.15***	35.22***	18.25***
Root length (mm)	29.95***	35.97***	4.26*	2.44
R: S length ratio	479.55***	3.95*	10.85**	3.67
SLA (cm ² mg ⁻¹)	14.97***	72.84***	4.62*	4.68**
SRL (cm mg ⁻¹)	0.22	17.60**	1.79	6.88**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

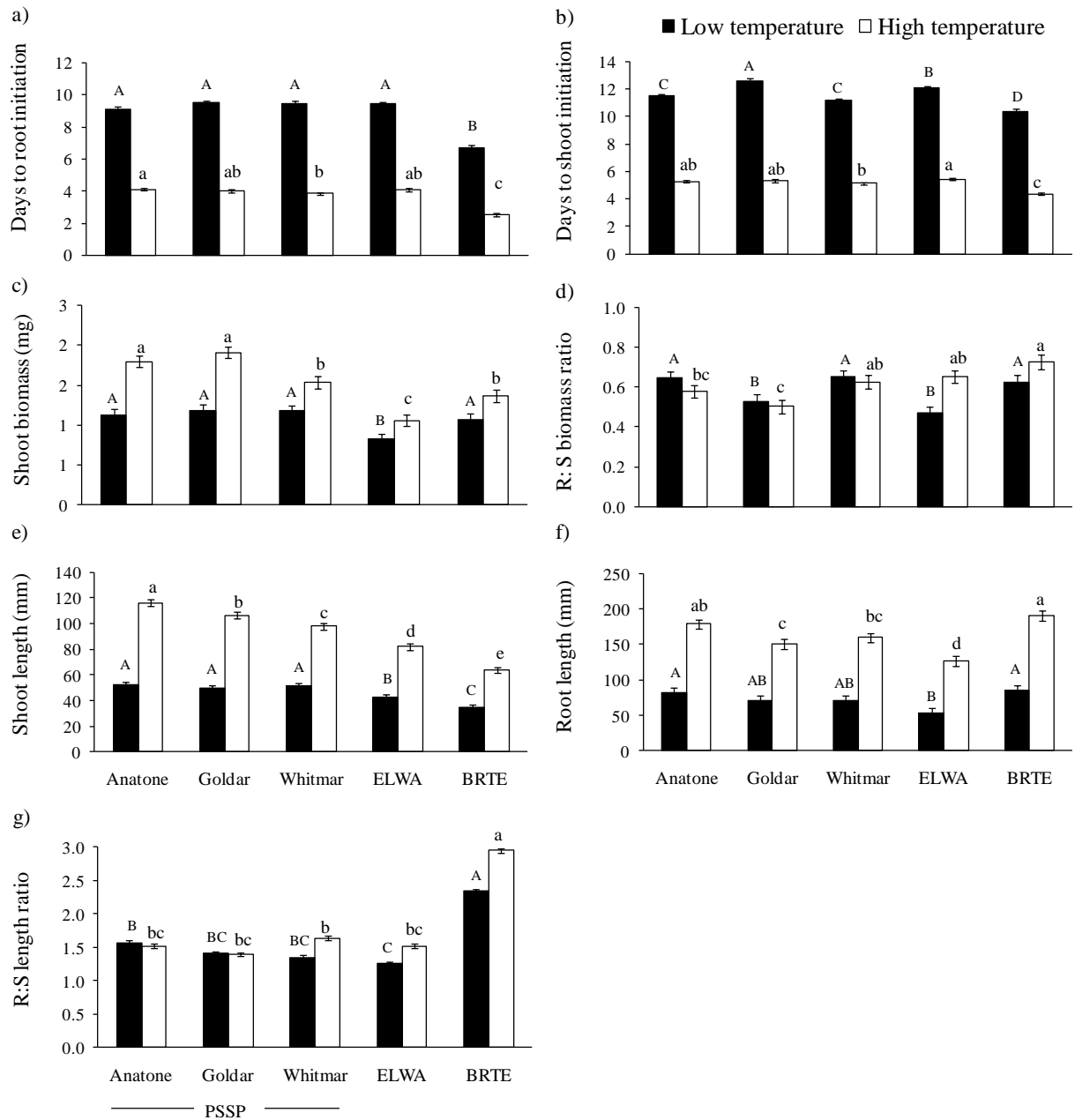


Figure 1. Means and standard errors for days to root initiation of *P. spicata* (PSSP), Secar *E. wawawaiensis* (ELWA), and *B. tectorum* (BRTE) at low and high temperatures. Comparison of means and standard errors for (a) days to root initiation, (b) days to shoot initiation, (c) shoot biomass, (d) root- to-shoot (R:S) biomass ratio, (e) shoot length, (f) root length, and (g) root-to-shoot (R:S) length ratio of three *P. spicata* (PSSP) populations, Secar *E. wawawaiensis* (ELWA), and *B. tectorum* (BRTE) at low and high temperatures. Different upper-case and lower-case letters represent significant ($P < 0.05$) differences among populations within low and high temperatures, respectively.

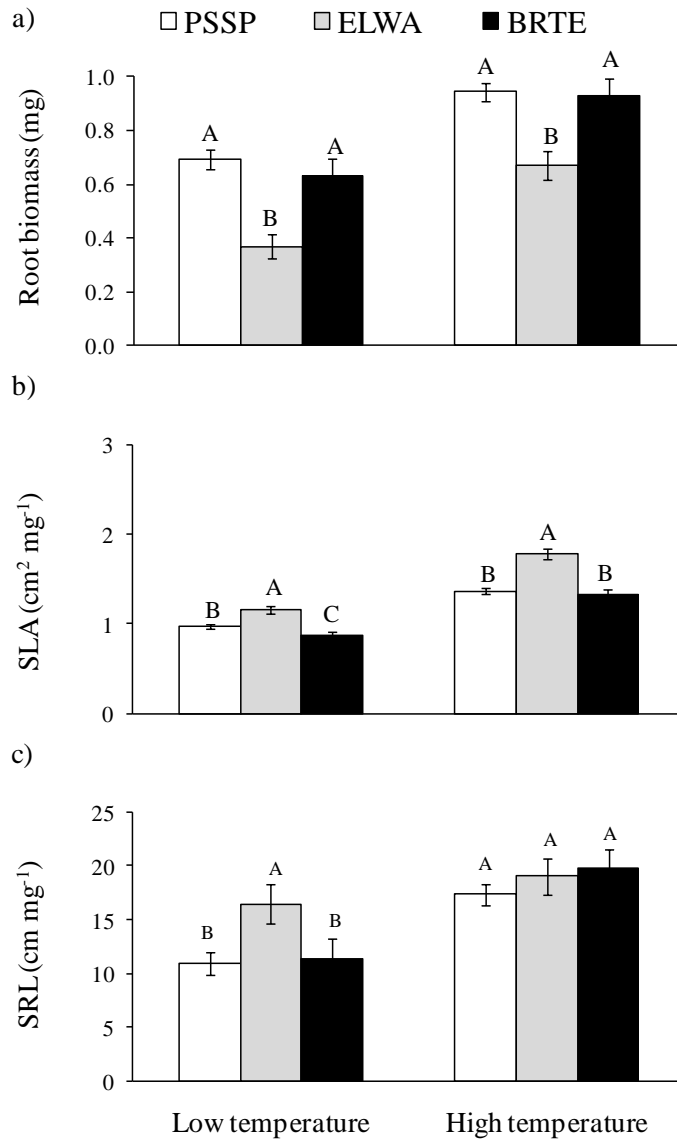


Figure 2. Comparison of means and standard errors for (a) root biomass, (b) specific leaf area (SLA), and (c) specific root length (SRL) of *P. spicata* (PSSP), *E. wawawaiensis* (ELWA), and *B. tectorum* (BRTE) at low and high temperatures. Different letters represent significant ($P < 0.05$) differences among populations within each temperature.

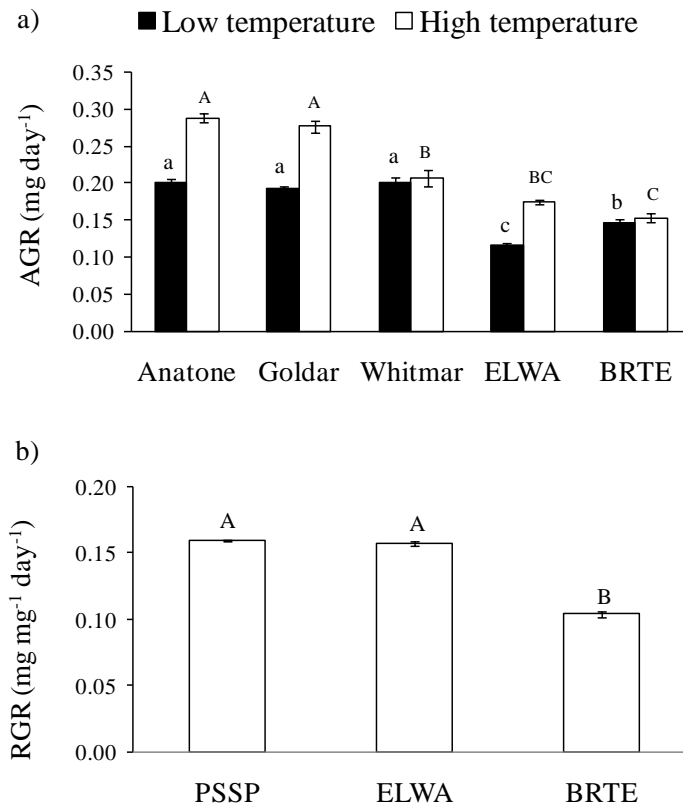


Figure 3. (a) Means and standard errors for absolute growth rate (AGR) of three *P. spicata* (PSSP) populations, Secar *E. wawawaiensis* (ELWA), and *B. tectorum* (BRTE) at low (lower case) and high (upper case) temperatures and (b) means and standard error for relative growth rate (RGR) across temperatures. Different letters represent significant ($P < 0.05$) differences within (a) and across (b) temperatures.

CHAPTER 3

SEED-MASS, SEEDLING MORPHOLOGICAL-TRAIT, AND GROWTH-RATE
VARIATION IN *PSEUDOROEGNERIA SPICATA*¹**Abstract**

Across-species relationships among seed mass, seedling morphological traits, and growth rates are well known in plant ecology, but within species, these classical relationships are not always sustained. In particular, the effects of fluctuating temperatures and the potential correlations between seed mass germination, seedling morphology, and growth are relatively unexplored. Here, we correlated seed mass with germination rates, seedling morphological traits, and absolute (AGR) and relative (RGR) growth rates under two temperature regimes across six diploid and three tetraploid populations of *Pseudoroegneria spicata*, a perennial Triticeae bunchgrass native to the Intermountain West, USA. Correlations between seed mass and plant traits were stronger at high temperature (20/15°C) than at low temperature (10/5°C). At the very-young seedling stage, we found no differences among populations for RGR and no association between RGR and either seed mass or specific leaf area (SLA). Ploidy level explained significant amounts of variation for most traits except root-to-shoot biomass ratio (R: S) and R: S length ratios, but trait differences attributed to ploidy were better explained by associated differences in seed mass. Lighter-seeded populations (all diploid) germinated and initiated shoots earlier and produced significantly greater SLA and specific root length. Heavier-seeded populations (mostly tetraploid) displayed greater shoot and root biomass, shoot length, and AGR. This suggests contrasting strategies of greater surface-

¹ Coauthored by: Jayanti Ray Mukherjee, TA Jones, PB Adler, and TA Monaco

area production by the lighter-seeded *P. spicata* populations and higher biomass accumulation by the heavier-seeded populations.

Introduction

Variation in seed mass acts as a major determinant of germination rate and seedling growth and establishment (Harper et al. 1970; Fenner and Thompson 2005). Owing to their greater reserves, larger seeds may display earlier germination, greater germination percentage, better seedling establishment, larger initial seedling mass, higher root-extension rates, and greater stress tolerance, both across species (Leishman and Westoby 1994; Kidson and Westoby 2000; Baraloto et al. 2005) and within species (Wulff 1973; Wulff 1986; Zhang and Muan 1990; Greipsson and Davy 1995; Baraloto et al. 2005). Nevertheless, an across-continent comparison of a large number of species suggests that small-seeded species germinate faster (Norden et al. 2009). Smaller-seeded species also typically display greater specific leaf area (SLA) and specific root length (SRL), which are traits associated with high relative growth rate (RGR) (Wright and Westoby 1999; Poorter and Garnier 2007). High RGR accounts for the ability of seedlings originating from smaller seeds to eventually catch up or surpass seedlings grown from larger seeds (Thomas 1966; Aparicio et al. 2002). While this strongly negative relationship between seed mass and RGR is well established among species, this relationship is seldom expressed across genotypes within species (Townsend and Wilson 1981; Meyer and Carlson 2001; Castro et al. 2008).

Contrasting diurnal temperature is one of the most critical requirements for germination (Thompson and Grime 1983; Gutterman 1993), and optimum ambient temperature during periods of soil-water availability is an important cue for seed

germination (Easton and Kleindorfer 2008). Species vary in their temperature requirements for germination (Fenner and Thompson 2005), and small-seeded species are known to have more stringent temperature requirements for germination compared to large-seeded species (Easton and Kleindorfer 2008). Low soil temperature can reduce root growth and reduce water-absorption capabilities in plants (Anderson and McNaughton 1973), and across species, temperature is known to indirectly impact morphological and physiological traits through its effect on growth. For example, SLA plays a dominant role in interspecific variability for RGR only at higher temperatures (Atkin et al. 2006), while such information is still lacking for within-species comparisons and at fluctuating temperatures. Temperature may also significantly affect initial seedling mass, initial leaf area, and RGR, as seen in the herbaceous legume *Astragalus cicer* L. (Townsend and Wilson 1981), which may obscure the relationship between seed mass and RGR.

Pseudoroegneria spicata (Pursh.) A. Löve is a C₃, perennial Triticeae bunchgrass that occurs throughout the North American Intermountain West (West 1991). Due to historical overgrazing practices, weed invasion, and increased fire frequency, *P. spicata* abundance has declined in much of its native range (Young and Longland 1996; DiTomaso 2000). The delayed germination of *P. spicata* in early spring relative to exotic winter-annual grasses that have invaded this region, and its inability to preempt abundant early-season soil moisture and nutrient resources, has contributed to its decline (Harris 1977; Young and Allen 1997). While root growth is essential to ensure seedling establishment in challenging rangeland environments (Svejcar 1990), low soil temperatures are known to hinder root growth of *P. spicata* (Harris and Wilson 1970).

Because of its presumed historic importance on Intermountain rangelands, *P. spicata* is commonly included in restoration seed mixes in the region (Monsen et al. 2004), and considerable resources have been devoted to providing more effective plant materials of this species (Asay et al. 2003).

Our objectives were 1) to compare *P. spicata* populations for germination traits (days to germination and shoot initiation), seedling morphological traits (shoot and root biomass, ratios of shoot and root biomass and length, SLA, and SRL), and seedling growth rates [absolute growth rate (AGR) and RGR] under high- and low-temperature regimes and 2) to characterize the relationships between seed mass, seedling-germination rates, seedling morphological traits, and seedling-growth rates among the populations under both temperature regimes.

Comparison of nine populations of *P. spicata* across two ploidy levels (diploid and tetraploid), which encompass a broad range for seed mass (Fig. 1), provide us an excellent opportunity to examine the above-mentioned relationships. Based on above mentioned literature, we predicted that heavier-seeded populations of *P. spicata* would germinate faster and display greater seedling biomass, seedling length, and AGR, while lighter-seeded populations would display greater SLA, SRL, and RGR. Cold temperature delays germination (Guterman 1993) and significantly reduces seedling growth (Townsend and Wilson 1981). Hence, we predicted that correlations of seed mass and seedling traits would be weaker at low temperature than at high temperature because seed mass is less likely to limit seedling biomass when growth is limited within a fixed time. In addition, we expected that the tetraploid *P. spicata* seedlings would be more productive than the diploid seedlings, but that diploid and tetraploid populations with

similar seed mass would perform similarly, as reported for naturally occurring populations of *Dactylis glomerata* L. by Bretagnolle et al. (1995).

Materials and methods

We employed three released commercial populations ('Goldar', 'Whitmar,' and Anatone germplasm) and six experimental populations (P-3, P-20t, P-22, P-24, P-27t, and T-17t) of *P. spicata* in this study. Of these nine populations, P-20t, P-27t, and T-17t are tetraploids ($2n = 28$), as indicated by the terminal 't', while the remainder are diploids ($2n = 14$). Both diploid and tetraploid populations of *P. spicata* occur naturally (Jensen et al. 1995). Brief descriptions of the development of the six experimental populations are provided here. Of the tetraploids, T-17t is naturally occurring (Steptoe Butte, Whitman Co., WA), P-27t was developed from a tetraploid experimentally induced from the diploid P-3 (a population developed by intermating several collections in the Grande Ronde Valley, Union Co. and Wallowa Co., OR), and P-20t was derived from a cross between the natural tetraploid T-17t and a tetraploid induced from P-3. The diploid P-22 population was developed from four cycles of selection on P-1 (origin unknown), and the diploid P-24 population was developed from three cycles of selection on Acc:238 (Lind, WA). To avoid confounded maternal effects resulting from multiple seed-production environments, seeds of all populations were produced in a common environment at Utah State University's Evans Farm at Millville, UT. While all seeds were produced in the same field and year, individual seed-production plots were established perpendicular to the prevailing wind and isolated by distance to minimize cross-pollination between plots. Seeds were harvested in 2007, cleaned, and refrigerated until experimental use. Seed mass was determined by weighing 36 lots of 100 seeds for each population (Fig. 1). In

our study, we refer to P-3, P-20t, P-27t, and T-17t as large-seeded populations and P-22, P-24, Goldar, Anatone, and Whitmar as small-seeded populations.

Two temperature regimes, high and low, were randomly assigned to two growth chambers (Percival Scientific, Inc., Perry, IA, USA). Actual temperatures for high (alternating 12-hour 20 ± 1.25 °C days/ 15 ± 2.16 °C nights) and low (10 ± 0.33 °C days/ 5 ± 0.28 °C nights) were recorded in each chamber using a data logger (Watchdog, Spectrum Technologies, Plainfield, IL, USA). The two temperature regimes were intended to represent typical early- and late-spring temperatures of the Intermountain West (Limbach and Call 1996).

The experimental design was a completely randomized design with subsamples. Seeds were germinated in germination pouches (CYG seed germination pouch, Mega International, St. Paul, MN, USA), which are folded plastic sheets enclosing a blotter paper. Four seeds were placed in each germination pouch, and 25 pouches (replicates) of each population were used in each growth chamber. Thus, a total of 100 seeds of each population were evaluated for each temperature.

To initiate the study, we sprayed the blotter papers to saturation with distilled water on 12 Aug. 2008, and pouches were rewatered as necessary throughout the experiment to maintain ideal moisture conditions and prevent drought stress. Each seed was monitored daily, and the dates of first initiation of radicle and coleoptile were recorded. Seedlings of 12 replicates were destructively harvested after 8 days for high temperature and 16 days for low temperature (early harvest). The low-temperature harvest was delayed to allow seedlings to be sufficiently sizeable to measure morphological traits. For both temperature regimes, a second harvest was taken seven

days following the first harvest on the remaining 13 replicates to permit calculation of growth rates. On their designated harvest dates, seedlings were scanned at 300 dots per square inch (46.5 dots per cm²) using WinRHIZO Pro Version 2005b (Reagent Instrument Inc., Québec City, Canada). After scanning, shoots and roots were separated, oven dried at 60°C for 48 hours, and weighed. Scanned images were analyzed for shoot and root length, leaf area, SLA, and SRL. Other than days to root and shoot initiation, all seedling traits were determined from the second harvest. Absolute growth rate (AGR) was calculated as $AGR = \mu[(W_2 - W_1)] / (t_2 - t_1)$, and relative growth rate was calculated as $RGR = \mu[(\ln W_2 - \ln W_1)] / (t_2 - t_1)$, where W_1 and W_2 were the final dry masses (shoot + root) from the early and late harvests and μ was the mean biomass for the indicated harvest. To determine if AGR and RGR differed among populations, we performed ANOVA on total biomass at two temperatures and included harvest in the model. A significant effect of harvest x population interaction for total biomass indicated that populations were significantly different for AGR and RGR (ln-transformed total biomass). Also a significant interaction for harvest x temperature x population was interpreted as a variation across temperatures.

Except for days to root and shoot initiation and growth rates, variables were measured at the second harvest. Data were analyzed using SAS 9.1.3 (SAS Institute 2003) and transformed as necessary to meet normality assumptions. Means were back-transformed for presentation. In addition, heterogeneous variances were corrected using a REPEATED (Littell et al. 1996) statement in PROC MIXED. This reduced within-temperature heterogeneity by partitioning the residual variance into separate groups for each temperature. For mean comparisons, one-way analysis of variance was performed in

PROC MIXED. Pearson's correlations among seedling traits were calculated across populations using PROC CORR. Because we had no replication of growth chambers and because the days to harvest for the two temperature regimes differed, we refrained from directly comparing high and low temperatures. However, we did assess the interaction of populations with temperature regimes for the seedling traits. To determine if accession differences could be accounted for by seed mass (linear and quadratic) or ploidy level, we fit these terms in alternate order, as well as their interaction, before accession in the model.

Results

The populations used in this study encompassed a 1.9-fold range for seed mass (Fig. 1). On average, tetraploid populations had heavier seeds ($6.0 \pm 0.2 \text{ mg seed}^{-1}$) than diploid populations ($4.3 \pm 0.02 \text{ mg seed}^{-1}$), ranging from $6.46 \text{ mg seed}^{-1}$ for the tetraploid population, P-27t, to $3.48 \text{ mg seed}^{-1}$ for the diploid, Whitmar. An exception was the high seed-mass diploid, P-3 ($6.18 \pm 0.02 \text{ mg seed}^{-1}$).

Ploidy level had a significant ($P < 0.01$) effect on the variation of all but two very-young seedling traits, R: S biomass ratio and R: S length ratio (Table 1). On average, diploids initiated roots slightly earlier (0.2 days) than the tetraploid populations. Diploid populations also initiated shoots slightly earlier than tetraploids, but this difference was more pronounced at low temperature (0.32 days) than high temperature (0.05 days), as exhibited by a significant temperature x ploidy-level interaction (Table 1). On average, tetraploid populations also had greater shoot biomass (18 %), root biomass (20 %), shoot length (8 %), and root length (12 %) than diploid populations (Table 1), while diploids had significantly greater SLA (5 %) and SRL (13 %) compared to tetraploids.

Across temperatures, the diploids P-24 and P-22 were the earliest populations to germinate, with the tetraploid P-27t and the diploids P-3 and Goldar being the slowest (Fig. 2a). No interaction between temperature and population was seen for days to root initiation (germination), but this interaction was significant for days to shoot initiation (Table 1). At low temperature, P-24, P-22, Whitmar, and Anatone initiated shoots earliest, while Goldar, P-3, and P-27t were latest. At high temperature, on the other hand, P-24 and T-17t were earliest to initiate shoots and the large-seeded populations P-3 and P-20t were latest (Fig. 3a).

Populations differed for shoot and root biomass, but they did not interact with temperature for this trait (Table 1). Across temperatures, P-20t and P-27t populations displayed the greatest shoot biomass, and Whitmar, P-24, and Anatone displayed the least shoot biomass (Fig. 2b). P-20t and P-27t also exhibited the greatest root biomass, while P-24, Goldar, Whitmar, P-22, and Anatone populations were lowest (Fig. 2c). Like shoot and root biomass, the R: S biomass ratios of the populations did not interact with temperature (Table 1; Fig. 2d). The lightest-seeded population, Whitmar, displayed highest R: S biomass ratio, while P-24 and Goldar were lowest for this trait.

Populations differed and interacted with temperature for shoot length (Table 1). P-20t displayed the greatest shoot length at both temperatures, but Whitmar performed especially poorly at low temperature relative to the other populations (Fig. 3b). Goldar, P-22, P-20t, and P-27t had greater root length than the other populations (Fig. 2e) without an interaction (Table 1). Populations differed and interacted with temperature for R: S length ratio (Table 1). At low temperature, P-3, P-20t, and Anatone were greater than the

other populations (Fig. 3c), while at high temperature, Whitmar, Anatone, P-24, P-20t and T-17t were greatest, and P-3 was lowest.

We found variation for SLA among populations to be only marginally significant ($P < 0.10$), and Whitmar's SLA was highest and similar to most of the populations, except P-3 and P-20t, which were the lowest (Fig. 2f). We did detect a small, but though highly significant ($P < 0.01$), difference for SLA between ploidy levels across temperatures, as mentioned above (Table 1). For SRL, on the other hand, populations both differed and interacted with temperature. For example, SRL of Goldar and Whitmar was 34% and 84% greater at high temperature than at low temperature, respectively (Fig. 3d). However, this difference was only 12% for P-3.

Absolute growth rate and RGR were calculated separately for each temperature. We found a significant interaction between harvests and populations for total biomass indicating that populations were significantly ($P < 0.005$) different for AGR, but not for RGR (ln-transformed total biomass). The lack of significance for the harvest x temperature x population interaction indicated that populations varied similarly across temperatures for both AGR and RGR. On average, P-20t ($0.299 \text{ mg day}^{-1}$) was highest for AGR, while Whitmar was lowest ($0.204 \text{ mg day}^{-1}$) (Fig. 2g).

Across temperatures, seed mass was positively correlated ($P < 0.05$) with shoot biomass, root biomass, and AGR, and to a lesser extent ($P < 0.10$) with days to root initiation, days to shoot initiation, and shoot length. On the other hand, seed mass was negatively correlated with SLA and SRL (Table 2, Fig. 4a, b). Seed mass correlations with seedling traits were generally greater at high temperature, which likely reflects the greater variation commonly associated with larger means. Absolute growth rate and seed

mass were positively correlated across temperatures, and correlations between AGR and individual seedling traits were similar to correlations between seed mass and those traits. We did not find significant ($P > 0.10$) variation among populations for RGR. Despite a positive correlation between RGR and AGR and a positive correlation between AGR and seed mass, there was no significant relationship ($P > 0.10$) between RGR and seed mass (Table 2).

Discussion

Most studies have examined the relationship between seed mass and germination at the within-population level rather than among populations. Variation for seed mass within populations has been generated by either manually sorting seeds, e.g., Winn (1985), Wulff (1986), Paz and Martínez-Ramos (2003), or by utilizing half-sib open-pollinated lines that vary for seed mass, e.g., Benard and Toft (2007), Castro et al. (2008). To the best of our knowledge, among-population studies have all obtained their seeds directly from wildland sites, e.g., Counts and Lee (1991), Greipsson and Davy (1995), Meyer and Carlson (2001), thereby possibly introducing a confounded maternal effect due to seed-production environment (Roach and Wulff 1987). To our knowledge, ours is the only population-level study of a non-crop species that has utilized seeds produced in a common environment.

In our study, seeds of nine *P. spicata* populations produced in a uniform environment did not support the hypothesis that larger seeds germinate faster. Instead, we found that lighter-seeded diploid *P. spicata* populations germinated faster than both heavier-seeded diploid and tetraploid populations under both temperature regimes, consistent with findings within (Maun and Cavers 1971) and across species (Jurado and

Westoby 1992; Norden et al. 2009). Seed mass and mean germination time are believed to be phylogenetically conserved traits, meaning that the range of variation within species for these traits is limited (Norden et al. 2009). Species with lighter seeds have minimal seed reserves for seedling growth and are more reliant on nutrients in their immediate soil environment (Easton and Kleindorfer 2008). Such species are known to be at a disadvantage in stressful and competitive environments (Jurado and Westoby 1992; Fenner and Thompson 2005). However, when soil nutrients are readily available following precipitation events, as in our study, it is likely that early germination associated with lighter seeds allows seedlings to become self-sufficient in terms of photosynthate production as quickly as possible.

We found that the heavy-seeded tetraploid and the heaviest-seeded diploid (P-3) populations of *P. spicata* displayed the greatest shoot biomass. A previous study with wildland-collected seeds of 47 *P. spicata* populations (Kitchen and Monsen 1994) reported no correlation between seed mass and germination rate, but larger-seeded populations did produce greater seedling shoot biomass, as in our study.

We found that heavier-seeded populations have greater AGR. In our study, the 85.6% difference in seed mass between Whitmar (lowest) and P-27t (highest) corresponded to a 68.9% difference in AGR. Relative growth rate, on the other hand, is growth rate per unit of initial biomass, and it is believed to be more appropriate for population comparisons than AGR (Hunt 1990). However, RGR did not vary among *P. spicata* populations, and we found no association between seed mass and RGR, similar to previous intraspecific studies (Wulff 1986; Tamet et al 1996; Castro et al. 2008). At the very-young seedling stage, seed size may be the major factor contributing to early growth

(Cideciyan and Malloch 1982); hence we were able to detect significant variation for AGR. But the effect of seed size on seedling size declines over time, and the size discrepancy between seedlings of heavier and lighter seeds typically diminishes with age (Kittock and Patterson 1962; Zhang and Muan 1990; Smart and Moser 1999). This is when variability in RGR becomes more important relative to AGR. Thus, our inability to detect RGR differences may be a result of our measurements being taken at the very-young seedling stage.

Seed mass was negatively associated with SLA and SRL, indicating that smaller-seeded populations invest preferentially in surface area above and below ground, while larger-seeded populations invest more in carbon per unit of surface area, a pattern similar to that reported for across-species comparisons (Lambers et al. 1998; Reich et al. 1998). But as RGR did not significantly vary among populations, we found no significant relationship between SLA and RGR, similar to what has been reported in other intraspecific studies (Wulff 1986; Vaughton and Ramsey 2001; Castro et al. 2008), but in contrast to many interspecific studies (Reich et al. 1998; Wright and Westoby 1999). In addition to the time when RGR was measured, this lack of association within species may also indicate that within species, RGR is a more conserved trait and that across species, factors other than seed mass may be major contributors to variation for RGR (Castro et al. 2008).

The negative relationship between seed mass, on the one hand, and SLA and SRL, on the other, suggests that light-seeded populations may be better suited for above- and below-ground resource acquisition. Species or populations that invest more in surface area and exhibit higher SLA and SRL are better able to forage for both above- and below-

ground resources, signifying an adaptation to nutrient-rich environments (Westoby 1998; Poorter and Jong 1999). Alternatively, species or populations that invest more in biomass and display low SLA and SRL have low resource-acquisition rates and generally originate in resource-poor environments (Reich et al. 2003). It must be emphasized that the above-cited studies refer to the distribution of species across environments that vary for nutrient resources, particularly nitrogen, and rarely refer to environmental variation for soil-moisture availability (Westoby 1998). Greater SRL has been associated with drought-stress environments (Poot and Lambers 2003), while low SLA and SRL have been generally associated with drought stress (Nicotra et al. 2002; Poorter and Garnier 2007).

In *Plantago media* L., a native perennial dicot from the aridlands of Eurasia, seed-size variation between diploids and tetraploids is only evident for artificially induced tetraploids (Van Dijk and Delden 1990). In our study, the two populations developed through chromosome doubling, P-27t and P-20t, were the most productive of all *P. spicata* populations. In induced tetraploids, gigantic size effects ('gigas-effects'; Stebbins [1971]) are believed to be associated with reduced fertility and are subsequently eliminated by selection pressure (Van Dijk and Delden 1990; Bretagnolle et al. 1995). However, among our materials, P-27t was developed from the diploid P-3 by chromosome doubling, yet these two populations had similar seed masses. In our study, shoot and root biomass and shoot and root length of P-3, the heaviest-seeded diploid, were more similar to the other tetraploids than to the remaining diploids, signifying that the variations in traits are better explained by seed mass than by ploidy. The commercially available *P. spicata* populations (Goldar, Anatone, and Whitmar), all being

light-seeded populations, displayed similar productivity and similar SRL at both temperatures. However, Anatone germplasm was the population that exhibited relatively high R: S length ratio at both temperatures.

In conclusion, a wide range for seed mass within *P. spicata* was associated with significant differences in germination rates, seedling morphological traits, and seedling AGR at the very-young seedling stage. Correlations of seed mass with growth rates and seedling morphological traits were stronger at high temperature, as we predicted. Contrary to our prediction, however, we found that lighter-seeded populations initiated roots and shoots earlier than heavier-seeded populations. We also found that heavier-seeded populations exhibited greater shoot and root biomass, shoot length, and AGR, likely because of greater seed reserves (Fenner and Thompson 2005). On the other hand, lighter-seeded populations displayed greater SLA and SRL, although root length was uncorrelated with seed mass.

The ability to germinate and successfully establish is paramount for a species like *P. spicata* to persist within its native range. Larger seeds within a species are known to generate seedlings of greater vigor (Kittock and Patterson 1962; Smart and Moser 1999), and in our study, heavier-seeded populations produced larger seedlings. Heavier-seeded populations were associated with lower SRL, yet whether low SRL is advantageous or disadvantageous under our semi-arid conditions is unclear (Ryser 2006). Are smaller-seeded *P. spicata* populations better or worse adapted to more arid environments than larger-seeded populations? While this study was not designed to answer this question, the light-seeded P-24 originated from the most arid (253 mm average annual precipitation [AAP]) and lowest-elevation (480 m) site, while the heavy-seeded T-17t originated from

the most mesic (639 mm AAP) and highest-elevation (1100 m) site. These two populations were similar for SRL at high temperature, but at low temperature, P-24 featured the highest SRL, and T-17t was near the bottom (Fig. 3c).

In Intermountain West ecosystems, high soil-resource availability is synchronous with cold temperature (Harris and Wilson 1970), and it is under these conditions that seedling establishment of perennial grasses takes place. If high SRL at low temperature, as displayed by P-24, is advantageous for more arid sites, then lighter-seeded populations may be preferred for successful seedling establishment in such drought-prone environments. Most restoration efforts in the Intermountain West take place in such environments, thus lighter-seeded populations may be most appropriate for such applications.

References

- Anderson JE, McNaughton SJ (1973) Effects of low soil temperature, photosynthesis, leaf relative water content, and growth among elevationally diverse plant populations. *Ecology* 54: 1220-1233
- Aparicio N, Villegas D, Araus JL, Blanco R, Royo C (2002) Seedling development by biomass as affected by size and morphology in durum wheat. *J Agri Sci* 139: 143-150. DOI: 10.1017_S0021859602002411
- Asay KH, Chattersen NJ, Jensen KB, Jones TA, Waldron BL, Horton WH (2003) Breeding improved grasses for semiarid regions. *Arid Land Res Manage* 17: 469-478. DOI: 10.1080/15324980390225548

- Atkin OK, Loveys BR, Atkinson LJ, Pons TL (2006) Phenotypic plasticity and growth temperature: understanding interspecific variability. *J Expt Bot* 57: 267-281.
DOI:10.1093/jxb/erj029
- Baraloto C, Forget PM, Goldberg DE (2005) Seed mass, seedling size, and neotropical tree seedling establishment. *J Ecol* 93: 1156-1166. doi: 10.1111/j.1365-2745.2005.01041.x
- Benard RB, Toft CA (2007) Effect of seed size on seedling performance in a long-lived desert perennial shrub (*Ericameria nauseosa*: Asteraceae). *Int J Plant Sci* 168:1027-1033. DOI: 10.1086/518942
- Bretagnolle F, Thompson JD, Lumaret R (1995) The influence of seed size variation on seed germination and seedling vigour in diploid and tetraploid *Dactylis glomerata* L. *Annals of Botany* 76: 607-615
- Castro J, Reich PB, Sanchez-Miranda A, Guerrero JD (2008) Evidence that the negative relationship between seed mass and relative growth rate is not physiological but linked to species identity: a within-family analysis of Scots pine. *Tree Physiol* 28: 1077-1082. DOI:10.1093/treephys/28.7.1077
- Cideciyan MA, Malloch AJC (1982) Effect of seed size on the germination, growth, and competitive ability of *Rumex crispus* and *Rumex obtusifolius*. *J Ecol* 70: 227-232
- Counts RL, Lee PF (1991) Germination and early seedling growth in some northern wild rice (*Zizania palustris*) populations differing in seed size. *Can J Botany* 69:689-696
- DiTomaso JM (2000) Invasive species in rangelands: species, impacts, and management. *Weed Technol* 48: 255-265

- Easton LC, Kleindorfer S (2008) Interaction effects of seed mass and temperature on germination of Australian species of *Frankenia* (Frankeniaceae). *Folia Geobot* 43: 383-396
- Fenner M, Thompson K (2005) The ecology of seeds. Cambridge University Press, Cambridge, UK
- Greipsson S, Davy J (1995) Seed mass and germination behavior in populations of the dune-building grass *Leymus arenarius*. *Ann Bot-London* 76: 493-501.
- Gutterman Y (1993) Seed germination in desert plants. Springer-Verlag, Heidelberg, Berlin
- Harper JL, Lovell PH, Moore KG (1970) The shapes and size of seeds. *Ann Rev Ecol Syst* 1: 327-356
- Harris GA (1977) Root phenology as a factor of competition among grass seedlings. *J Range Manage* 30: 172-177
- Harris GA, Wilson A (1970) Competition for moisture among seedlings of annual and perennial grasses as influenced by root elongation at low temperature. *Ecology* 51: 530-534
- Hunt R (1990) Basic growth analysis: plant growth analysis for beginners. Kluwer Academic Publishers, Unwin Hyman, London, UK
- Jensen K, Curto M, Asay K (1995) Cytogenetics of Eurasian bluebunch wheatgrass and their relationship to North American bluebunch and thickspike wheatgrass. *Crop Sci* 35: 1157-1162
- Jurado E, Westoby M (1992) Seedling growth in relation to seed size among species among Arid Australia. *J Ecol* 80: 407-416

- Kidson R, Westoby M (2000) Seed mass and seedling dimensions in relation to seedling establishment. *Oecologia* 125: 11-17
- Kitchen SG, Monsen SB (1994) Germination rate and emergence success in bluebunch wheatgrass. *J Range Manage* 47: 145-150
- Kittcock DL, Patterson JK (1962) Seed size effects on performance of dryland grasses. *Agron J* 54: 277-278
- Lambers H, Chapin SF, Pons TL (1998) *Plant physiological ecology*. Springer, New York, USA
- Leishman MR, Westoby M (1994) The role of seed size in seedling establishment in dry soil conditions – experimental evidence from semi-arid species. *J Ecol* 82: 249-258
- Littell RC, Milleken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. SAS Institute Inc., North Carolina, USA
- Limbach WE, Call CA (1996) Germination response of Russian wildrye to variations in seed mass at fluctuating temperatures. *J Range Manage* 49: 346-349
- Maun MA, Cavers PB (1971) Seed production and dormancy in *Rumex crispus* L. The effects of removal of cauline leaves at anthesis. *Can J Bot* 49: 1123-1130
- Meyer SE, Carlson SL (2001) Achene mass variation in *Ericameria nauseosus* (Asteraceae) in relation to dispersal ability and seedling fitness. *Funct Ecol* 15: 274-281
- Monsen SB, Stevens R, Shaw NL (2004) Restoring western ranges and wildlands. U.S. Forest Service Gen. Tech. Rep. RMRS-GTR 136 (Vol. 2) Fort Collins, Colorado, USA

- Nicotra AB, Babicka N, Westoby M (2002) Seedling root anatomy and morphology: an examination of ecological differentiation with rainfall using phylogenetically independent contrasts. *Oecologia* 130: 136-145. DOI 10.1007/S004420100788
- A.B.
- Norden N, Daws MI, Antoine C, Gonzalez MA, Garwood NC, Chave J (2009) The relationship between seed mass and mean time to germination for 1037 tree species across five tropical forests. *Funct Ecol* 23: 203-210. DOI: 10.1111/j.1365-2435.2008.01477.x
- Paz H, Martinez-Ramos M (2003) Seed mass and seedling performance between eight species of *Psychotria* (Rubiaceae). *Ecology* 84: 439-450. DOI: 10.1890/0012-9658(2003)084[0439:SMASPW]2.0.CO;2
- Poorter H, Garnier E (2007) Ecological significance of inherent variation in relative growth rate and its components. In: Pugnaire FI, Valladares F (eds) *Functional plant ecology*. CRC Press, Boca Raton, FL, pp 67-100
- Poorter H, Jong RD (1999) A comparison of specific leaf area, chemical composition and leaf construction costs of field plants from 15 habitats differing in productivity. *New Phytol* 143: 163-176
- Poot P, Lambers H (2003) Are trade-offs in allocation pattern and root morphology related to species abundance? a congeneric comparison between rare and common species in the south-western Australian flora. *J Ecol* 91: 58-67. DOI: 10.1046/j.1365-2745.2003.00738.x
- Reich PB, Tjoelker MG, Walters MB, Vanderklein DW, Buschena C (1998) Close association of RGR, leaf and root morphology, seed mass and shade tolerance in

- seedlings of nine boreal tree species grown in high and low light. *Functional Ecology* 12: 327-338
- Reich PB, Wright IJ, Cavender-Bares J, Craine JM, Oleksyn J, Westoby M, Walters MB (2003) The evolution of plant functional variation: traits, spectra, and strategies. *Int J Plant Sci.* 164: S143-S164. DOI: 10.1086/374368
- Roach DA, Wulff RD (1987) Maternal effects in plants. *Ann Rev Ecol Syst* 18: 209-235
- Ryser P (2006) The mysterious root length. *Plant Soil* 286: 1-6
- SAS Institute (2003) SAS/STAT user's guide, SAS Institute version 9.1, Cary, North Carolina, USA
- Smart AJ, Moser LE (1999) Switchgrass seedling development as affected by seed size. *Agron J* 91: 335-338
- Stebbins GL (1971) Chromosomal evolution in higher plants. Arnold, London
- Svejcar TJ (1990) Root length, leaf area, and biomass of crested wheatgrass and cheatgrass seedlings. *J Range Manage* 43: 446- 448
- Tamet V, Boiffin J, Dürr C, Souty N (1996). Emergence and early growth of an epigeal seedling (*Daucus carota* L.): influence of soil temperature, sowing depth, soil crusting and seed mass. *Soil Till Res* 40: 25-38
- Thomas RL (1966) The influence of seed weight on seedling vigor in *Lolium perenne*. *Ann Bot-London* 30: 111-121
- Thompson K, Grime JP (1983) A comparative study on germination responses of diurnally-fluctuating temperatures. *J Appl Ecol* 20:141-156
- Townsend CE, Wilson AM (1981) Seedling Growth of Cicer Milkvetch as Affected by Seed Weight and Temperature Regime. *Crop Sci* 21: 405-409

- Van Dijk P, Delden WV (1990) Evidence for autotetraploidy in *Plantago media* and comparisons between natural and artificial cytotypes concerning cell size and fertility. *Hereditas* 65: 349-357
- Vaughton G, Ramsey M (2001) Relationships between seed mass, seed nutrients, and seedling growth in *Banksia cuminnghamii* (Proteaceae). *Int Plant Sci* 3: 599-606
- West NE (1991). Intermountain deserts, shrub steppes, and woodlands. In: Barbour MG and Billings WD (eds) *North American terrestrial vegetation*. Cambridge University Press, New York, USA
- Westoby M (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant Soil* 199: 213-227
- Winn AA (1985) The effects of seed size and microsite on seedling emergence in four populations of *Prunella vulgaris*. *J Ecol* 73: 831-840
- Wright I, Westoby M (1999) Differences in seedling growth behavior among species: trait correlation across species, and trait shifts nutrient compared to rainfall gradients. *J Ecol* 87: 85-97
- Wulff RD (1973) Intrapopulational variation in the germination of seeds in *Hyptis suaveolens*. *Ecology* 54: 646-649
- Wulff RD (1986) Seed size variation in *Desmodium paniculatum*: II. Effects on seedling growth and physiological performance. *J Ecol* 74: 99-114
- Young JA, Allen FL (1997) Cheatgrass and range science: 1930–1950. *J Range Manage* 50:530-535
- Young JA, Longland WS (1996) Impacts of alien plants on Great Basin rangelands. *Weed Technol* 10: 384-391

Zhang J, Muan MA (1990) Effects of sand burial on seed germination, seedling emergence, survival, and growth of *Agropyron psammophilum*. Can J Bot 68: 304-310

Table 1. Least-squares means for two ploidy levels and analysis of variance F -values for 10 seedling traits of nine populations of *P. spicata*.

	Model with ploidy level				Model without ploidy level	
	Between ploidy levels	Among 2x populations	Among 4x populations	Temperature* ploidy level	Population	Temperature* population
<i>Numerator df</i>	1			1	8	8
Days to root initiation	12.39**	6.60	6.79	0.02	7.18***	1.30
Days to shoot initiation	10.08**	8.49	8.67	5.36*	15.03***	5.66***
Shoot biomass (mg)	39.45**	1.48	1.74	2.87 ⁺	6.38***	1.62
Root biomass (mg)	34.61***	0.87	1.04	1.71	5.23***	0.88
R: S biomass ratio	2.47	0.59	0.61	1.61	4.62***	1.22
Shoot length (mm)	16.24***	79.69	86.48	1.89	4.22**	1.67
Root length (mm)	16.41***	114.34	127.60	0.68	3.44**	1.83 ⁺
R: S length ratio	0.17	1.47	1.49	0.93	2.90**	4.54***
SLA (cm ² mg ⁻¹)	10.35**	1.10	1.05	2.1	1.72 ⁺	0.87
SRL (cm mg ⁻¹)	13.22**	13.33	11.82	2.02	5.56***	2.29*
AGR (mg day ⁻¹) ‡	11.44**	0.226	0.270	0.34	2.40*	1.10
RGR (mg mg ⁻¹ day ⁻¹) ‡	0.67	0.150	0.158	0.07	0.80	0.69

⁺P < 0.10, * P < 0.05, **P < 0.01, ***P < 0.0001.

‡ significant differences in AGR and RGR among populations were estimated from the interaction of harvest*populations for total biomass (log-total biomass for RGR), and their variation across temperatures were estimated from the interaction of temperature*harvest*populations for the same trait.

Table 2. Correlations of 10 seedling traits with seed mass and absolute growth rate (AGR) under low and high temperature regimes.

Traits	Seed mass			AGR		
	Low temp.	High temp.	Across temps.	Low temp.	High temp.	Across temps
Root initiation	0.48	0.72*	0.60 ⁺	0.28	0.70*	0.47
Shoot initiation	0.60 ⁺	0.67*	0.60 ⁺	0.15	0.57	0.55
Shoot biomass	0.64 ⁺	0.84**	0.83**	0.95**	0.86**	0.91**
Root biomass	0.62 ⁺	0.91**	0.82**	0.95**	0.78*	0.88**
R: S biomass	0.38	-0.01	0.25	0.59 ⁺	-0.31	0.2
Shoot length	0.61 ⁺	0.61 ⁺	0.67 ⁺	0.85**	0.86**	0.88**
Root length	0.58	-0.09	0.19	0.84**	0.24	0.58
R: S length	0.45	-0.54	-0.33	0.65 ⁺	-0.45	-0.06
SLA	-0.44	-0.63 ⁺	-0.73*	-0.63 ⁺	-0.64 ⁺	-0.64 ⁺
SRL	-0.65 ⁺	-0.87*	-0.90**	-0.81**	-0.49	-0.73*
RGR	0.10	0.06	0.11	0.71*	0.76*	0.62 ⁺
AGR	0.59 ⁺	0.65 ⁺	0.74*			

⁺P < 0.10, * P < 0.05, **P < 0.01, ***P < 0.0001.

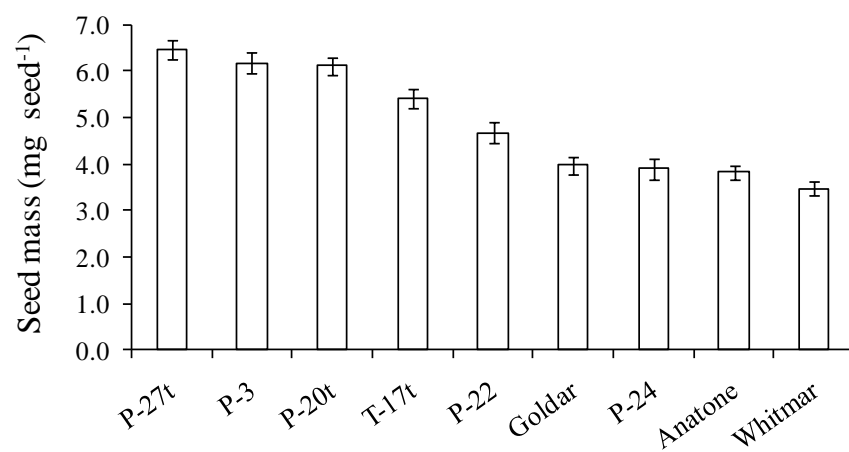


Figure 1. Means and standard deviations for seed mass of nine populations of *P. spicata*.

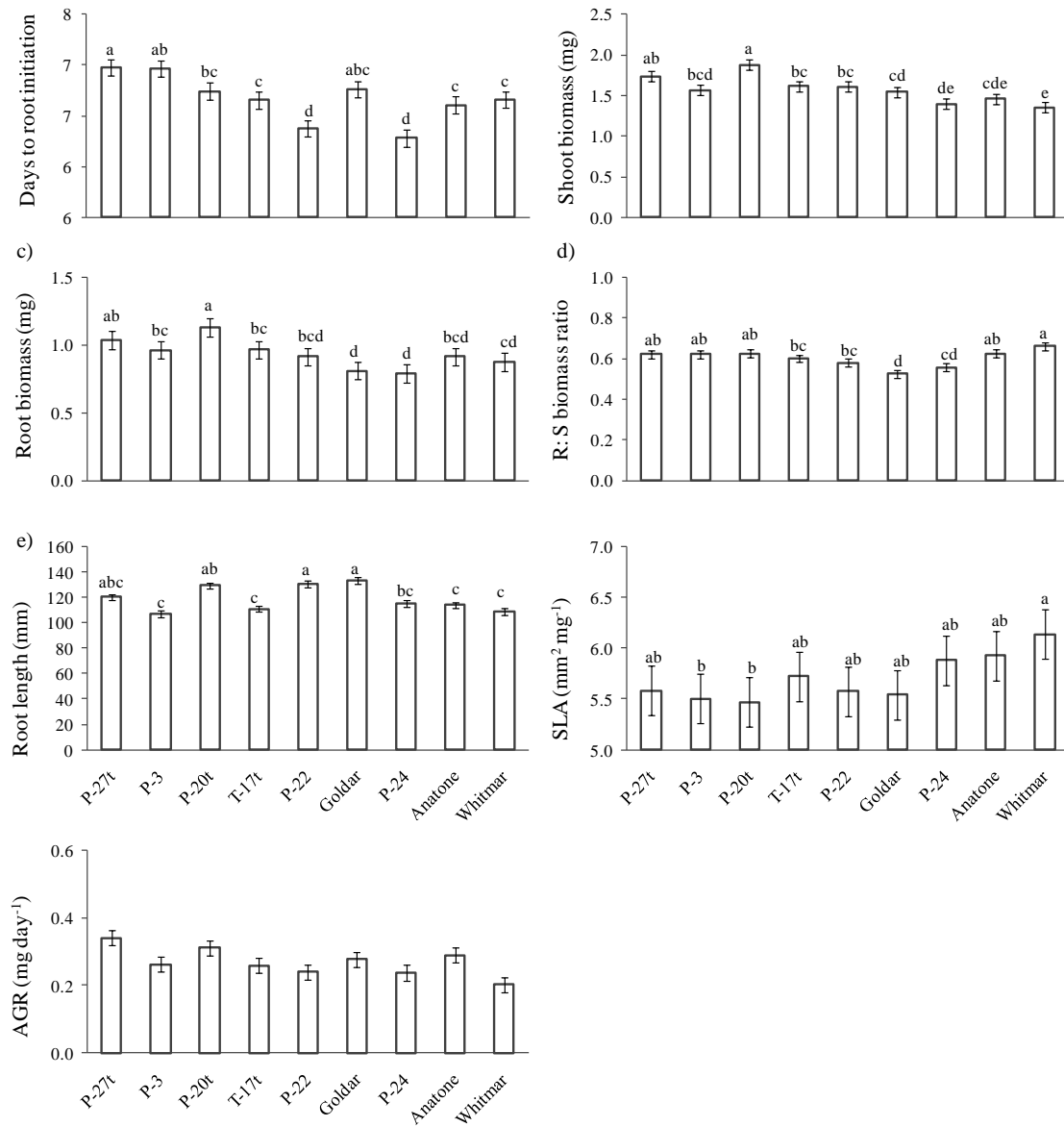


Figure 2. Means and standard errors for (a) days to root initiation, (b) shoot biomass, (c) root biomass, (d) root: shoot biomass ratio, (e) root length, (f) specific leaf area (SLA), and (g) absolute growth rate (AGR) of nine *P. spicata* populations. Different letters represent significant ($P < 0.05$) differences among populations for morphological traits, except AGR.

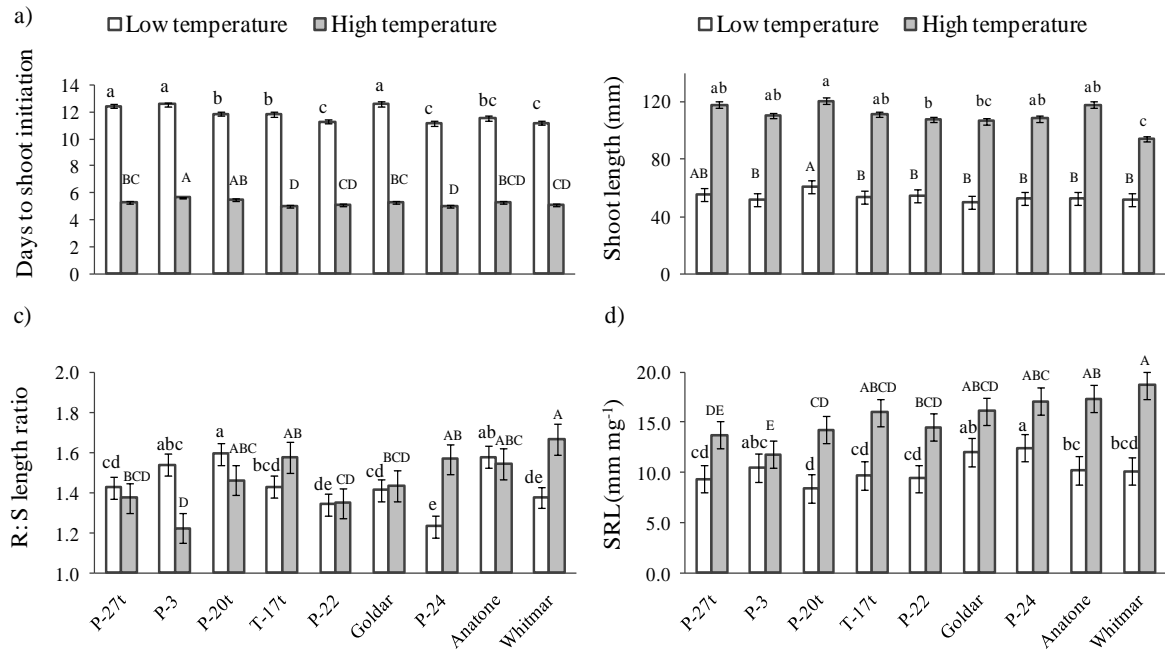


Figure 3. Means and standard errors for (a) days to shoot initiation, (b) shoot length, (c) R: S length ratio, and (d) specific root length (SRL) of nine *P. spicata* populations at low and high temperatures. Different lower-case and upper-case letters represent significant ($P < 0.05$) differences among populations within low and high temperatures, respectively.

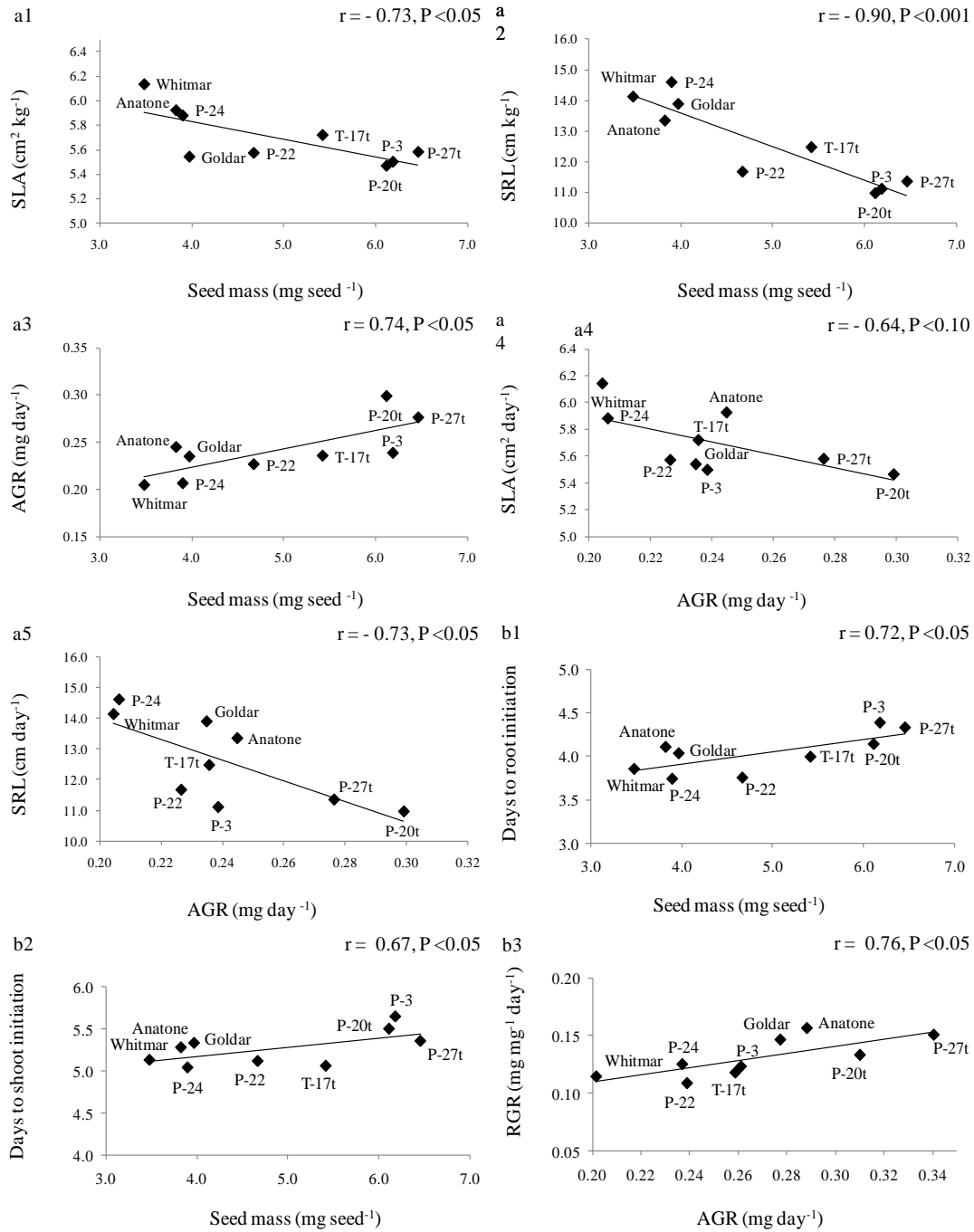


Figure 4. Correlations among nine *P. spicata* populations for seedling traits (a) across temperatures and (b) at high temperature.

CHAPTER 4

DROUGHT TOLERANCE IN TWO PERENNIAL BUNCHGRASSES USED FOR
RESTORATION IN THE INTERMOUNTAIN WEST, U.S.A.¹**Abstract**

An ideal restoration species for the semi-arid Intermountain West, USA would be one that grows rapidly when resources are abundant in the spring, yet tolerates summer's drought. We compared two perennial C₃, Triticeae Intermountain-native bunchgrasses, the widely occurring *Pseudoroegneria spicata* and the much less widespread *Elymus wawawaiensis*, commonly used as a restoration surrogate for *P. spicata*. Specifically, we evaluated seedlings of multiple populations of each species for biomass production, water use, and morphological and physiological traits that might relate to drought tolerance under three watering frequencies in a greenhouse. Shoot biomass of *E. wawawaiensis* exceeded that of *P. spicata* regardless of watering frequencies. At low watering frequency, *E. wawawaiensis* displayed 38% greater shoot biomass, 80% greater specific leaf area (SLA), and 32% greater precipitation use efficiency (PUE). One *E. wawawaiensis* population, E-46, displayed particularly high root biomass and water consumption at high watering frequency. We suggest that such a plant material could be especially effective for restoration of Intermountain rangelands by preempting early-season weeds for spring moisture and also achieving high PUE. Our data explain how *E. wawawaiensis* has been so successful as a restoration surrogate for *P. spicata* and highlight the importance of measuring functional traits such as PUE and SLA when characterizing restoration plant materials.

¹ Coauthored by: Jayanti Ray Mukherjee, TA Jones, PB Adler, and TA Monaco

Introduction

Plant functional traits may play an important role in native-plant restoration planning by characterizing species' responses to environmental stress (Naeem 2006). For example, a suite of interrelated morpho-physiological traits may reveal the mechanisms underlying drought tolerance. Slow-growing species with low specific leaf area (SLA, leaf surface area per unit biomass) are known to be more stress tolerant (Lambers et al. 1998). Specific leaf area is considered to be the best single predictor of relative growth rate (Chapin et al. 1993; Hunt and Cornelissen 1997; Poorter and Van der Werf 1998; Westoby et al. 1998; Grime 2001; James and Drenovsky 2007; Poorter and Garnier 2007), and rapidly growing species with higher SLA are known to produce greater shoot biomass under high resource availability. Drought tolerance is also favored by a high specific root length (SRL, root length per unit biomass), indicating an increase in belowground absorptive surface area (Ryser 2006). However, rapidly growing species also feature traits that can be disadvantageous under drought conditions, such as low precipitation use efficiency (PUE), high stomatal conductance (g_s), and low root: shoot ratio (R:S) (Ryser and Lambers 1995; Fernandez and Reynolds 2000; Poorter and Garnier 2007).

Frequent droughts and competition from invasive annual grasses constrain native-perennial seedling establishment in the arid and semi-arid rangelands of North America's Intermountain West (Daubenmire 1942; Harris 1967; Harris and Wilson 1970; Young and Allen 1997). *Pseudoroegneria spicata* (Pursh.) A. Löve (bluebunch wheatgrass) and *Elymus wawawaiensis* J. Carlson & Barkworth (Snake River wheatgrass) are perennial, C₃ Triticeae bunchgrasses native to Intermountain West rangeland ecosystems.

Pseudoroegneria spicata generally occurs in medium- to coarse-textured soils from foothills to mid-montane habitats and is widespread in the region (Ogle 2002a). Due to annual grass invasion and resultant wildfires, restoration efforts have accelerated in the region (Monsen et al. 2004), and this species is prominent in seed mixes used for this purpose.

While *E. wawawaiensis* and *P. spicata* occur on similar soils, the former possesses a much more restricted distribution (Carlson and Barkworth 1997). Furthermore, due to a superficial morphological resemblance, *E. wawawaiensis* was taxonomically confused with *P. spicata* prior to being shown to be genomically distinct (Carlson and Barkworth 1997). In the meantime, ‘Secar’ *E. wawawaiensis* has become widely used a surrogate for *P. spicata* in restoration applications. Interestingly, the popularity of *E. wawawaiensis* in restoration seeding mixes has continued (Lambert 2006), despite its description as a new species. This is likely because in restoration practices, Secar establishes better and is generally considered to possess greater productivity and superior drought tolerance than commercially available *P. spicata* plant materials (Morrison and Kelley 1981; Carlson and Barkworth 1997; Young and Allen 1997; Ogle 2002b; Monsen et al. 2004; Lambert 2006).

While drought tolerance of *E. wawawaiensis* is reputed to be greater than *P. spicata* (Ogle 2002b), the relative effect of drought on growth and physiological response of the two species has not been characterized. We investigated the effect of experimental drought on functional traits associated with drought tolerance in four populations of *P. spicata* and two populations of *E. wawawaiensis* in a greenhouse. Along with biomass production and total water use by the species, we examined six additional plant traits:

SLA, SRL, PUE, R:S, mid-day xylem pressure potential (Ψ), and g_s . Our objective was to compare multiple populations of the two species for productivity at three water levels and to identify traits that might be responsible for drought tolerance in these grasses. In addition, we wished to test the validity of the reputation of *E. wawawaiensis* as the more drought tolerant of the two species, which would justify its use as a surrogate for *P. spicata* in restoration practice. We hypothesized that *E. wawawaiensis* would display greater drought tolerance and levels of traits associated with drought tolerance, such as lower SLA, higher R: S, higher SRL, less negative Ψ , lower g_s , and higher PUE.

Materials and Methods

Materials for this study included four *P. spicata* populations and two *E. wawawaiensis* populations. The *P. spicata* populations were ‘Goldar’ (originally from the Umatilla National Forest, WA, 1000-1200 mm average annual precipitation), Anatone germplasm (Anatone Valley, WA, 250 – 500 mm), P-22 (developed from the P-1 population, origin unknown), and P-26 (developed from P-7 *P. spicata*, a commercially released multiple-origin polycross germplasm). The *E. wawawaiensis* populations were ‘Secar’ (Lewiston, ID, 200 – 300 mm) and E-46 (developed from populations originating at 10 different locations in ID and WA). Of these six populations, Goldar, Anatone, P-7 (all *P. spicata*), and Secar (*E. wawawaiensis*) are commercially released populations used in contemporary restoration, while the remainder are experimental populations.

Plastic pots (23 cm in height and 8 cm in diameter) were filled with 2,450 g of a 3:1 mix of Kidman fine sandy loam (coarse-loamy, mixed, mesic Calcic Haploxerolls) and Ricks gravelly loam (coarse-loamy, mesic Calcic Haploxerolls). Water-holding capacity of the pots (pots of similar size and equal amount of soil mix) was standardized

using the gravimetric method (Israelson and West 1922), in which five pots with four drainage outlets apiece were watered to saturation. These pots were covered with paper sheets to preclude evaporation, drained, and weighed every five minutes. Once drainage ceased, final weights averaging 3,010 g were recorded. Percentage relative water content was calculated as: $(W_{\text{wet}} / W_{\text{Dry}}) \times 100$, where W_{wet} is the weight of wet soil and W_{Dry} is the weight of dry soil.

The experiment was conducted at the USDA-ARS Forage and Range Research Laboratory greenhouse at Utah State University, Logan, Utah. Fifty seeds of each population were germinated on blotter paper one week before initiation of the experiment. After one week, on 13 June 2006, 21 seedlings of similar size of each population were transplanted to pots. The pots had been filled with steam-sterilized soil mixed as described above, except they had no drainage holes in order to maintain them at water-holding capacity for each treatment.

We imposed three levels of drought stress by manipulating watering frequency based on a preliminary experiment modified from the protocol of Sack and Grubb (2002). Tap water was used for all watering frequencies, which were imposed within a week of initiation of the study. The high watering frequency (HF) treatment was watered every 4-7 days (18 times in 12 weeks), moderate watering frequency (MF) every 10-12 days (8 times), and low watering frequency (LF) every 17-21 days (5 times). The LF treatment was designed to reduce growth without resulting in plant mortality. The three watering frequency treatments represent a range in pulse frequency for early spring and summer precipitation (Sack and Grubb 2002). Schwinning et al. (2003) demonstrated that cold-

desert plants take up water in direct proportion to overall precipitation amount with little effect due to the magnitude of individual precipitation events.

The greenhouse temperature ranged from 18 to 31 °C, slightly lower than natural summer temperatures in Logan. Immediately following transplanting, pots were watered to 3,010 g, which was previously determined to be field capacity. Pots were rewatered to reach 3,010 g when the total pot mass fell to 2,840 g for the HF treatment, 2,640 g for the MF treatment, and 2,510 g for the LF treatment (Fig. 1). At the beginning of the experiment, the HF, MF, and LF treatments corresponded to 15.9%, 7.8%, and 2.4% water contents, respectively. The experiment was conducted for 12 weeks under natural day-length conditions under a shade cloth. Pots were fertilized with Miracle-Gro (20N-20P₂O₅-20K₂O with all micronutrients) along with watering (560 ml for each pot, 4.2 g/L) at the initiation of the experiment.

In determining the pot mass for rewatering each treatment, as described above, we did not adjust for increasing fresh weight of the seedlings as the experiment proceeded. As a result, water contents at rewatering declined relative to initial water content (field capacity) as the experiment proceeded and the plants grew. However, based on calculations we made with biomass measured at the end of the experiment when the effect would be the largest, this bias appears to be small. The difference in water content between pots of high- and low-biomass accessions was 0.2% for the LF treatment and 1.3% for the HF treatment. Furthermore, this small amount of bias was on the conservative side, making differences we reported smaller than they actually were.

A total of 378 pots were arranged in a randomized complete block design with three watering frequencies, six populations, and 21 replications as blocks, plus nine

control pots. Watering frequencies, species, and population within species were considered to be fixed effects, and replications were considered random. Data were analyzed using PROC MIXED in SAS (2003). We used CONTRAST statements to calculate significant difference among populations within the two species and we employed ESTIMATE statements to calculate the interaction of watering frequency and populations within species.

Stomatal conductance was measured at 11.00 to 13.00 hours during the last week before harvest on two leaves per plant plant using a steady-state leaf porometer (SC-1, Decagon Devices, Inc., Pullman, Washington). In the same week, mid-day xylem pressure potential was measured using a Scholander pressure chamber (PMS Instruments Co., Corvallis, OR, USA). Xylem pressure potential was measured on a single fully-green leaf, no more than one minute later than leaf excision. We used a sharp razor blade to incise fully green leaves across the midrib, and 95% of the leaf surface was then inserted it into the slit-seal rubber stub. Pressure was then applied to the stub until the appearance of xylem solution through the cut end of the leaf, and this pressure was recorded (Boyer 1995). Stomatal conductance and Ψ were measured on 15 of 21 replicates, just before rewatering was required for each treatment. Above-ground and below-ground biomass were subsequently harvested and dried (60° C for 3 days), R:S was calculated, and the experiment was terminated on 2 September 2006. To calculate SLA, a sub-sample of five fresh leaves was fed through a LI-3100C leaf-area meter (LI-COR leaf-area meter, Lincoln, Nebraska), after which the leaves were dried (60° C for 3 days) and weighed. We subsampled six replicates to determine SRL. Roots were extracted, cleaned thoroughly under flowing water, scanned using WinRHIZO Pro Version 2005b

(Reagent Instrument Inc., Québec City, Canada), and analyzed for total root length.

Specific root length was calculated as total root length divided by root biomass. Total water used by an individual plant was calculated as water added across all watering dates minus water lost through evaporation through the same date, as determined by the control pots. Precipitation use efficiency of productivity was calculated by dividing total biomass (g) by total water added (kg) for each individual (Kramer and Boyer 1995; Huxman et al. 2004).

Results

Plant mortality was similar for HF [7.1 ± 3.1 (SE) %], MF ($4.8 \pm 3.0\%$) and LF ($7.1 \pm 3.5\%$). Low watering frequency made mid-day xylem pressure potential (Ψ) more negative (Table 1, Fig. 2a), indicating our treatments were effective in generating variation for drought stress. However, Ψ did not differ among species or populations within species, and neither interacted with WF (Table 1).

Averaged over watering frequencies and populations, *Elymus wawawaiensis* produced 22% greater shoot biomass than *P. spicata* (Table 1, Fig. 3a). *Pseudoroegneria spicata* populations differed in shoot biomass, with Goldar being highest, but *E. wawawaiensis* populations did not (Table 1, Fig. 3b). When averaged over watering frequencies and populations, *E. wawawaiensis* also produced 35% greater root biomass than *P. spicata* (Table 1, Fig. 3c). Averaged over watering frequencies, *P. spicata* populations differed in root biomass, while *E. wawawaiensis* populations did not (Table 1). Despite this trend, we found a significant interaction between populations of both species and watering frequency for root biomass (Table 1). Specifically, this resulted because P-26 (78%) and Goldar (76%) *P. spicata* showed greater reduction in root

biomass from HF to LF than P-22 (66%) and Anatone (68%) (Fig 3d), as did E-46 *E. wawawaiensis* (81%) relative to Secar (61%). This suggests that P-26, Goldar, and E-46 displayed more phenotypic plasticity for the response of root biomass to soil moisture (Fig. 3d).

Across species, moderate drought (from HF to MF) reduced total biomass by 30%, while severe drought (high to low WF) caused 64% reduction of total biomass. Across watering frequencies and populations, *Elymus wawawaiensis* produced 24% greater total biomass than *P. spicata* (Table 1, Fig. 3e). The *P. spicata* populations did not interact with watering frequency for total biomass (Table 1), but an interaction of *E. wawawaiensis* populations with watering frequency (Table 1) resulted because E-46 was able to produce higher root biomass at HF relative to Secar (Fig. 3d), rather than to any differences in shoot-biomass production (Fig. 3b).

Elymus wawawaiensis exhibited 24% greater R:S than *P. spicata* at HF (Table 1), while under MF and LF, both species were similar for this trait (Fig. 4a). Among *P. spicata* populations, Anatone and Goldar displayed greater R:S ratio than P-22 and P-26 when averaged across watering frequencies (Fig. 4b). A significant interaction between *E. wawawaiensis* populations and watering frequencies for R:S ratio (Table 1) resulted from an exceptionally high R:S ratio of E-46 at HF (Fig. 4c), which resulted from this population's exceptionally plastic root-biomass production (Fig. 3d).

Elymus wawawaiensis produced 37% greater SLA than *P. spicata* under HF, while this difference grew to 54% and 80% under MF and LF, respectively (Fig 5a), generating a watering frequency x species interaction (Table 1). However, populations within each of these species responded similarly to watering frequency for SLA (Table

1). While SLA of all *P. spicata* populations was quite similar averaged across WFs, E-46 *E. wawawaiensis* displayed 12% greater SLA than Secar (Table 1, Fig. 5b). Specific root length was not affected by watering frequency (Table 1), although *E. wawawaiensis* displayed 15% greater SRL than *P. spicata* (Table 1, Fig. 5c). P-22 had the lowest SRL of the *P. spicata* populations (Table 1, Fig. 5d), while the *E. wawawaiensis* populations were similar for this trait.

Compared to HF, MF and LF reduced g_s by 47% and 63%, respectively (Table 1, Fig. 2b). Averaged across watering frequencies, g_s of P-26 *P. spicata* was considerably higher than the other five populations (Fig. 2c). Averaged across watering frequencies and populations, *P. spicata* used 5.8% less water than *E. wawawaiensis* (Table 1, Fig. 6a). The *P. spicata* populations varied in their water use, and they also interacted with watering frequency for this trait (Table 1, Fig. 6b). At HF, P-22 used less water than the other *P. spicata* populations, while *P. spicata* populations were similar for water use at MF and LF (Fig. 6b). Between *E. wawawaiensis* populations, Secar used 10% less water than E-46 at HF, while like *P. spicata* populations, their water use was similar at MF and LF (Fig. 6b). Precipitation use efficiency was highest for HF, followed by MF and LF (Table 1, Fig. 7a), and across watering frequencies, *E. wawawaiensis* was 20% greater for PUE than *P. spicata* (Table 1, Fig. 7b). Across watering frequencies, Goldar exhibited significantly higher PUE than P-22 and P-26, with Anatone being intermediate, while the two *E. wawawaiensis* populations were similar to Goldar (Fig. 7c).

Discussion

In our study, *E. wawawaiensis* produced greater shoot biomass than *P. spicata* under both drought and non-drought conditions. Therefore, based on the plant materials we evaluated, we consider *E. wawawaiensis* to be the more drought tolerant of the two species. If a species is more productive regardless of watering frequencies, it will likely be more successful in difficult Intermountain West environments. In spite of its greater drought tolerance, *E. wawawaiensis* displayed greater SLA, a trait associated with high growth rate under favorable conditions and drought susceptibility. In fact, SLA of *E. wawawaiensis* increased relative to *P. spicata* at LF.

For these species, then, there appears to be no trade-off between growth potential and drought tolerance (Fernandez and Reynolds 2000; Grime 2001), although it is possible that inclusion of a more stressful “killing treatment” would have permitted the detection of such a trade-off. The lack of trade-off does not deny the validity of the classical trade-off hypothesis. Rather, a trade-off may lie within some other trait combination, for example, between drought tolerance and defense or survival or between mechanisms of drought tolerance and those of drought resistance (Chapin et al. 1993; Haugen et al. 2008).

While we expected that *E. wawawaiensis*, due to its high SLA, would display lower PUE under drought, we found the opposite to be the case at MF and LF. Although several studies have shown that drought increases PUE and decreases SLA (Wright et al 1994; Craufurd et al. 1999; Xu and Zhou 2008; Songsri et al. 2009), in our study, drought reduced both PUE and SLA in *P. spicata* and reduced PUE, but not SLA, in *E. wawawaiensis*. Although there was a positive trend, SLA and PUE were not significantly

correlated across watering frequencies. A classical hypothesis in plant ecophysiology would be that PUE increases with drought, but studies showing a higher PUE with increasing drought stress are generally a consequence of mild experimental stress (Ramirez et al. 2008). In our study, however, PUE decreased with increased experimental drought, which is more likely to be the typical ecological response under severe drought conditions (Songsri et al. 2009). Similar to Songsri et al. (2009), we measured PUE of productivity, a measurement integrated over the duration of the experiment, as opposed to instantaneous PUE. The former measurement is preferable for rangeland plants, as optimal use of water over time involves optimal distribution of stomatal opening along the gradient of aridity (Jones 1992).

The trait combination of high water use under non-limiting conditions and high PUE under limiting conditions may be favored in pulse-regulated arid and semi-arid ecosystems (Goldberg and Novoplansky 1997). In light of this principle, a desirable plant material would have greater water use at HF and high PUE at LF, which provides balance between productivity and water conservation (Jones 1992). In our study, E-46 *E. wawawaiensis* used the greatest amount of water at HF and, along with Secar *E. wawawaiensis*, also had the highest PUE at LF. Among the *P. spicata* populations, Goldar best displayed this trait combination.

Of the two *E. wawawaiensis* populations, E-46 displayed higher root biomass at HF, which may have been facilitated by its high SLA relative to Secar. At HF, E-46 also exhibited higher R:S and water use, yet at LF, E-46 was similar to Secar for these traits. These data suggest that E-46 is better suited to take advantage of resource pulses than Secar, yet just as well suited to the drought conditions of the inter-pulse period. The

superior growth of E-46 plants under non-limiting conditions may allow it to capitalize on and competitively preempt soil resources when they are most abundant in the spring, thereby enhancing its prospects for survival through the difficult first summer of establishment (Goldberg and Novoplansky 1997).

We also found that *E. wawawaiensis*, the species with higher SLA and greater shoot biomass, tended to have lower g_s regardless of watering frequencies. Although the difference in g_s between the two species was primarily due to one population, P-26 *P. spicata*, this high- g_s population also exhibited low SLA, indicating a negative association between these traits. High g_s could be a strategy that *P. spicata* employs to lower leaf temperature in order to avoid heat stress and photo-inhibition (Kappen and Valladares 2007; Pereira and Chaves 1993), as *P. spicata* has fewer seedling leaf hairs compared to *E. wawawaiensis* (Jones et al. 1991). Recent studies have shown that plant architecture may play an important role in conserving water by influencing micro-climatic conditions (Ryel et al. 1993, Ramirez et al. 2008). Lax-leaved (high SLA) species, such as *E. wawawaiensis*, may be able to maintain cool leaf temperatures by self-shading, thereby reducing water losses by transpiration and increasing PUE (Ramirez et al. 2008). We believe that plant materials like E-46 *E. wawawaiensis*, which express high water use in the pulse period and high PUE in the interpulse period, are more likely to be successful for restoration applications in water-limited pulse-regulated environments, particularly in the face of competition from invasive plants. In our study, Secar *E. wawawaiensis* sustained the smallest reduction of mean root biomass at LF, followed by P-22 and Anatone *P. spicata*, while Goldar and P-26 *P. spicata* and E-46 *E. wawawaiensis* showed greater reductions in mean root biomass, reflecting greater phenotypic plasticity. Species

with greater phenotypic plasticity are generally considered to be less drought tolerant (Fernandez and Reynolds 2000, Grime 2001), but E-46 produced as much shoot and root biomass as Secar at MF and LF. High SRL confers greater root absorptive surface area under drought (Ryser 2006), but in our study LF reduced root biomass without reducing SRL. Maintenance of SRL in response to drought, despite a decrease in root biomass, could be a successful strategy for survival in water-limited pulse-regulated environments.

In summary, two species once thought to be taxonomically synonymous, showed significant differences for functional traits and growth rates. If we define a highly productive genotype as one that maintains high productivity under both drought and non-drought conditions, then *E. wawawaiensis* is the more productive of the two species. Although claims have previously been made that Secar *E. wawawaiensis* is relatively more drought resistant in comparison to *P. spicata* (Morrison and Kelley 1981; Asay et al. 2001), this study is the first to provide an explanation of its drought tolerance based on functional traits. While we correctly hypothesized that *E. wawawaiensis* would be the more drought tolerant of the two species, our predictions regarding associated functional traits were only partially correct. While *E. wawawaiensis* tended to be favored with high SRL, low g_s , and high PUE, it still displayed high SLA. The finding that *E. wawawaiensis* has greater SLA than *P. spicata* helps to explain the former's higher shoot biomass.

This study forms a baseline for understanding drought-tolerance mechanisms in native perennial Triticeae bunchgrasses. Together, these results help to explain how *E. wawawaiensis* has come to be a widely and successfully used restoration surrogate for *P. spicata* in restoration plantings since Secar's release in 1980. Water use, PUE, SLA, and

SRL are functional traits that deserve to be emphasized when evaluating species and developing new plant materials for the purpose of rangeland restoration. Quantitative studies conducted under field conditions that examine relative growth rates with respect to functional traits are needed to verify our findings.

References

- Asay KH, Horton WH, Jensen KB, Palazzo AJ (2001) Merits of native and introduced Triticeae grasses on semiarid rangelands. *Can J Plant Sci* 81:45-52.
doi.org/10.1080/713936115
- Boyer, JS (1995) Measuring the water status of plants and soils. Academic Press, San Diego, California, USA
- Carlson JR, Barkworth ME (1997) *Elymus wawawaiensis*: a species hitherto confused with *Pseudoroegneria spicata* (Triticeae, Poaceae). *Phytologia* 83:312-330
- Chapin FS III, Autumn K, Pugnaire F (1993) Evolution of suites of traits in response to environmental stress. *Am Nat* 142: S78-S92
- Craufurd PQ, Wheeler TR, Ellis RH, Summerfield RJ, Williams JH (1999) Effect of temperature and water deficit on water-use efficiency, carbon isotope discrimination, and specific leaf area in peanut. *Crop Sci* 39:136-142
- Daubenmire RF (1942) An ecological study of the vegetation of southeastern Washington and adjacent Idaho. *Ecol Monogr* 12:53-79
- Fernandez RJ, Reynolds JF (2000) Potential growth and drought tolerance of eight desert grasses: lack of a trade-off? *Oecologia* 123:90-98
- Goldberg D, Novoplansky A (1997) On the relative performance of competition in unproductive environments. *J Ecol* 85:409-418

- Grime JP (2001) Plant strategies, vegetation processes, and ecosystem properties. Wiley, Chichester, UK
- Harris GA, Wilson AM (1970) Competition for moisture among seedlings of annual and perennial grasses as influenced by root elongation at low temperature. *Ecology* 51:530-534
- Harris GA (1967) Some competitive relationships between *Agropyron spicatum* and *Bromus tectorum*. *Ecol Monogr* 37:89-11
- Haugen R, Steffes L, Joy W, Paul B, Steven M, Siemens DH (2008) Evolution of drought tolerance and defense: dependence of trade-offs on mechanisms, environment, and defense switching. *Oikos* 117:231-244 doi: 10.1111/j.2007.0030-1299.16111.x
- Hunt R, Cornelissen JHC (1997) Components of relative growth rate and their interrelations in 59 temperate plant species. *New Phytol* 135:395-415
- Huxman TE, Smith MD, Fay PA, Knapp AK, Shaw MR, Loik ME, Smith SD, Tissue DT, Zak JC, Weltzin JF, Pockmann WT, Sala OE, Haddad BM, Harte J, Kock GW, Schwinning S, Small EE, Williams DG (2004) Convergence across biomes to a common rain-use efficiency. *Nature* 429: 651-654. doi:10.1038/nature02561
- Israelson OW, West FL (1922) Water holding capacity of irrigated soils. *Utah Aes Res Bull* 183: 1-24
- James JJ, Drenovsky RE (2007) A basis for relative growth rate differences between native and invasive forb seedlings. *Rangeland Ecol Manage* 60:395-400
- Jones HG (1992) Plants and microclimate: a quantitative approach to environmental plant physiology. Cambridge University Press, New York, USA

- Jones TA, Nielson DC, Carlson JR (1991) Development of a grazing-tolerant native grass for revegetating bluebunch wheatgrass sites. *Rangelands* 13:147-150
- Kappen L, Valladares F (2007) Opportunistic growth and desiccation tolerance: the ecological success of poikilohydrous autotrophs. In: Pugnaire FI, Valladares F (ed) *Functional plant ecology*. CRC Press, Boca Raton, Florida, USA, pp 7-66
- Kramer PJ, Boyer JS (1995) *Water relations of plants and soils*. Academic Press, San Diego, California, USA
- Lambers H, Chapin FS III, Pons TL (1998) *Plant physiological ecology*. Springer-Verlag, New York, USA
- Lambert S (2006) Seed use by Bureau of Land Management. Available via DIALOG <http://www.nps.gov/plants/meetings/mohave2006/Lambert-Mohave2006.pdf>. Accessed 23 Jan 2009
- Monsen SB, Stevens R, Shaw NL (2004) *Restoring western ranges and wildlands*. U. S. Forest Service. Gen. Tech. Rep. RMRS-GTR 136 (Vol. 2) Fort Collins, Colorado, USA
- Morrison KJ, Kelley CA (1981) *Secar bluebunch wheatgrass*. EB 0991. Washington State University, Cooperative Extension, Pullman, Washington, USA
- Naeem S (2006) Biodiversity and ecosystem functioning in restored ecosystem: extracting principles for a synthetic perspective. In: Falk DA, Palmer MA, Zelder JB (eds) *Foundation of restoration ecology*. Island Press, Washington, D.C., USA
- Ogle D (2002a) Plant fact sheet: bluebunch wheatgrass. Available via DIALOG http://plants.usda.gov/factsheet/pdf/fs_pssp6.pdf . Accessed 15 Oct 2009

Ogle D (2002b) Plant fact sheet: Snake River wheatgrass. Available via DIALOG.

http://plants.usda.gov/factsheet/pdf/fs_elwa2.pdf . Accessed 15 Oct 2009

Pereira JS, Chaves MM (1993) Plant water deficits in Mediterranean ecosystems. In:

Smith JAC, Griffiths H (ed) Plant water deficits: plant responses from cell to community. BIOS Scientific Publishers, Oxford, UK, pp 237-248

Poorter H, Van der Werf A (1998) Is inherent variation in RGR determined by LAR at low irradiance and by NAR at high irradiance? A review of herbaceous species.

In: Lambers H, Poorter H, Van Vuuren MMI (eds) Inherent variation in plant growth, physiological mechanisms and ecological consequences. Backhuys, Leiden, The Netherlands, pp 309-336

Poorter H, Garnier E (2007) Ecological significance of inherent variation in relative growth rate and its components. In: Pugnaire FI, Valladares F (eds) Functional plant ecology. CRC Press, Boca Raton, Florida, USA, pp 67-100

Ramirez DA, Valladares F, Blasco A, Bellot J (2008) Effect of tussock size and soil water content on whole plant gas exchange in *Stipa tenacissima* L.: extrapolating from leaf versus crown structure. *Env Exp Bot* 62:376-388.

doi:10.1016/j.envexpbot.2007.10.012

Ryel RJ, Beyschlag W, Caldwell MM (1993) Foliage orientation and carbon gain in two tussock grasses as assessed with a new whole-plant gas-exchange model. *Funct Ecol* 7: 115-124

Ryser P, Lambers H (1995) Root and leaf attributes for the performance of fast and slow-growing grasses at different nutrient supply. *Plant Soil* 170: 251-265

- Ryser P (2006) The mysterious root length. *Plant Soil* 286:1-6. doi: 10.1007/s11104-006-9096-1
- Sack L, Grubb PJ (2002). The combined impact of deep shade and drought on the growth and biomass allocation of shade-tolerant woody seedlings. *Oecologia* 131:175-185. doi: 10.1007/s00442-002-0873-0
- SAS (2003) SAS/STAT user's guide, SAS Institute version 9.1, Cary, North Carolina, USA
- Schwinning S, Starr BI, Ehleringer JR (2003) Dominant cold desert shrubs do not partition warm season precipitation by event size. *Oecologia* 136:252-260. DOI 10.1007/s00442-003-1255-y
- Songsri P, Jogloy S, Holbrook CC, Kesmala T, Vorasoot N, Akkasaeng C, Patanothai A (2009) Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. *Agr Water Manage* 96: 790-798. doi:10.1016/j.agwat.2008.10.009
- Westoby M, Cunningham S, Fonseca CR, Overton JM, Wright IJ (1998) Phylogeny and variation in light capture area deployed per unit investment in leaves: Designs for selecting study species with a view to generalizing. In: Lambers H, Poorter H, Van Vuuren MMI (eds) *Inherent variation in plant growth, physiological mechanisms and ecological consequences*. Backhuys, Leiden, The Netherlands, pp 539-566
- Wright GC, Nageshwara Rao RC, Farquhar GD (1994) Water use efficiency and carbon isotope discrimination in peanut under water deficit conditions. *Crop Sci* 34:92-97

Xu Z, Zhou G (2008) Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *J Exp Bot* 59:3317-3325.
doi:10.1093/jxb/ern185

Young JA, Allen FL (1997) Cheatgrass and range science: 1930–1950. *J Range Manage* 50:530-535

Table 1. Analysis of variance (*F*-values) for six morphological and four physiological traits for four *P. spicata* (PSSP) and two *E. wawawaiensis* (ELWA) populations for three watering frequencies (WF).

Traits / Effects <i>df</i>	WF 2	Species 1	PSSP populations 3	ELWA populations 1	WF * species 2	WF * PSSP populations 6	WF * ELWA populations 2
<i>Morphological traits</i>							
Shoot biomass (g)	113.57***	16.71***	5.69**	0.23	2.42	2.00	0.64
Root biomass (g)	109.70***	19.46***	8.93***	1.62	0.45	2.24*	6.13**
Total biomass (g)	65.20***	12.67**	4.32**	1.09	0.40	1.86	4.48*
Root : shoot	76.36***	7.17**	12.00**	16.62*	6.48**	0.94	16.41***
SLA (m ² kg ⁻¹)	5.86**	170.58***	2.22	4.65*	5.47**	0.71	2.19
SRL (mm mg ⁻¹)	0.96	4.95*	7.16**	0.08	2.42	1.45	1.05
<i>Physiological traits</i>							
Ψ (MPa)	22.80***	0.01	0.72	0.66	0.03	1.90	0.94
Total water use (L)	553.66***	15.60***	6.02**	2.03	2.79	2.96**	6.83**
g _s (mmoles m ² s ⁻¹)	38.93***	15.91***	3.93**	0.27	0.50	1.01	1.89
PUE (g kg ⁻¹)	33.41***	25.70***	4.82**	0.00	1.66	1.31	0.00

* *p* < 0.05, ** *p* < 0.01, *** *p* < 0.0001.

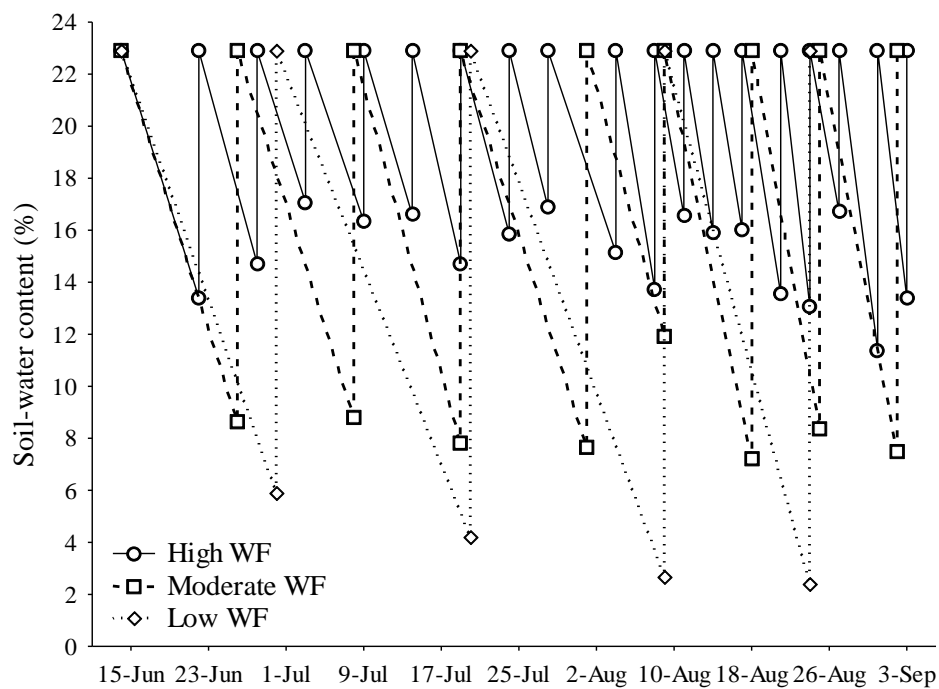


Figure 1. Soil-water content at three watering frequencies (WF) through a 12-week greenhouse experiment. Points denote dates that pots were watered to water-holding capacity.

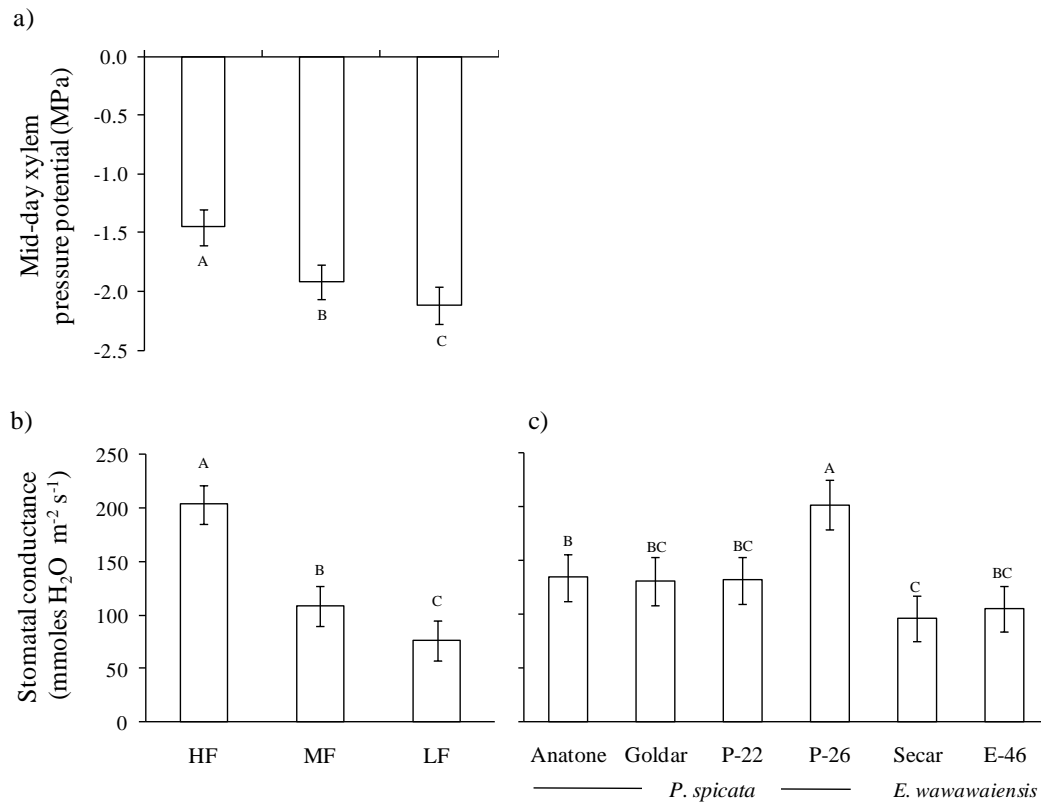


Figure 2. Means and standard errors for (a) mid-day xylem pressure potential at high (HF), moderate (MF), and low (LF) watering frequencies averaged across six populations, (b) stomatal conductance at three watering frequencies averaged across six populations, and (c) stomatal conductance of four *P. spicata* (PSSP) and two *E. wawawaiensis* (ELWA) populations averaged across three watering frequencies. Different letters represent significant ($P < 0.05$) differences among means.

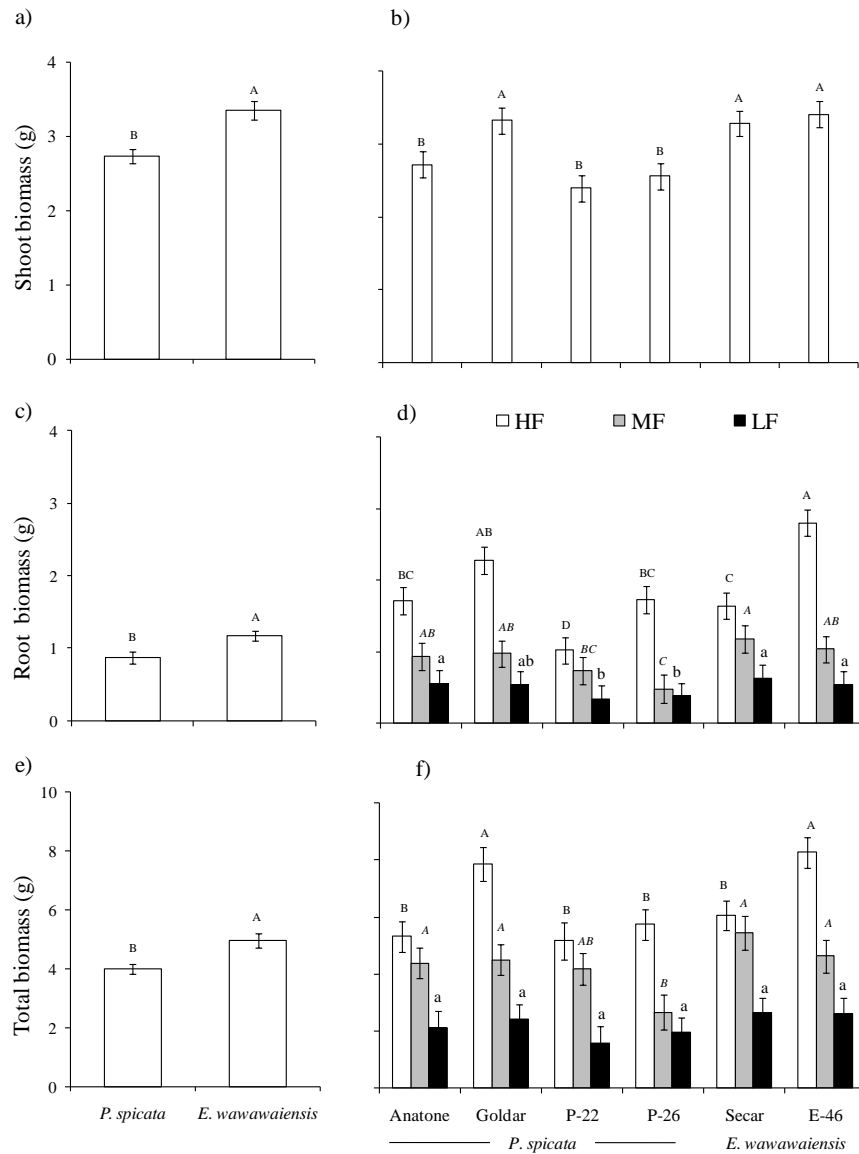


Figure 3. Means and standard errors for (a) shoot biomass of *P. spicata* and *E. wawawaiensis* averaged across their respective populations and high (HF), moderate (MF), and low (LF) watering frequencies (b) shoot biomass of four *P. spicata* and two *E. wawawaiensis* populations averaged across three watering frequencies, (c) root biomass of *P. spicata* and *E. wawawaiensis* averaged across their respective populations and watering frequencies, (d) root biomass of four *P. spicata* and two *E. wawawaiensis* populations at watering frequencies, (e) total biomass of *P. spicata* and *E. wawawaiensis* averaged across their respective populations and watering frequencies, and (f) total biomass of four *P. spicata* and two *E. wawawaiensis* populations at watering frequencies. Fig. 3a, b, c, e: Different letters represent significant ($P < 0.05$) differences among means. Fig. 3d, f: Different letters represent significant ($P < 0.05$) differences among populations within high (upper case), medium (upper-case italics), and low (lower case) watering frequencies.

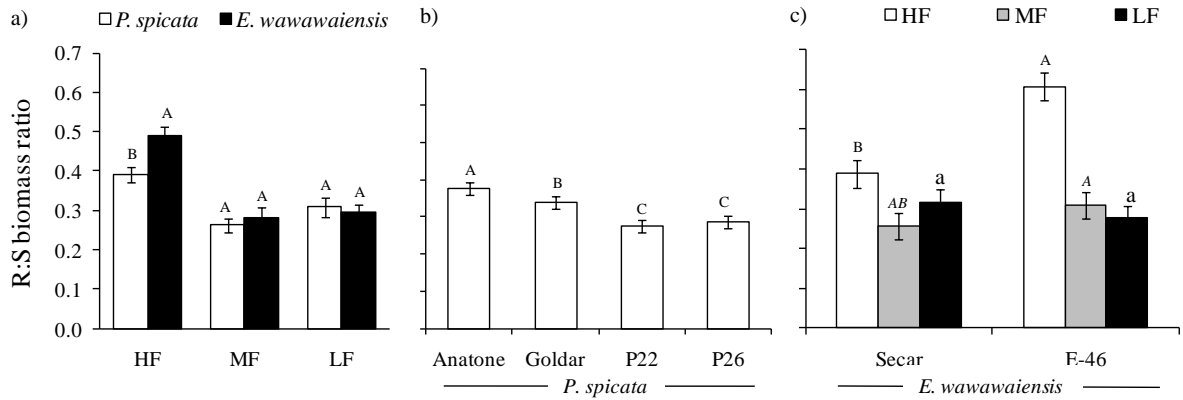


Figure 4. Means and standard errors for R:S biomass ratio for (a) *P. spicata* and *E. wawawaiensis* averaged across their respective populations at high (HF), moderate (MF), and low (LF) watering frequencies, (b) four *P. spicata* populations averaged across three watering frequencies, and (c) two *E. wawawaiensis* populations at HF, MF, and LF. Different letters represent significant ($P < 0.05$) differences (a) between species within watering frequencies, (b) among *P. spicata* populations, and (c) among *E. wawawaiensis* populations within HF (upper case), MF (upper-case italics), and LF (lower case) watering frequencies.

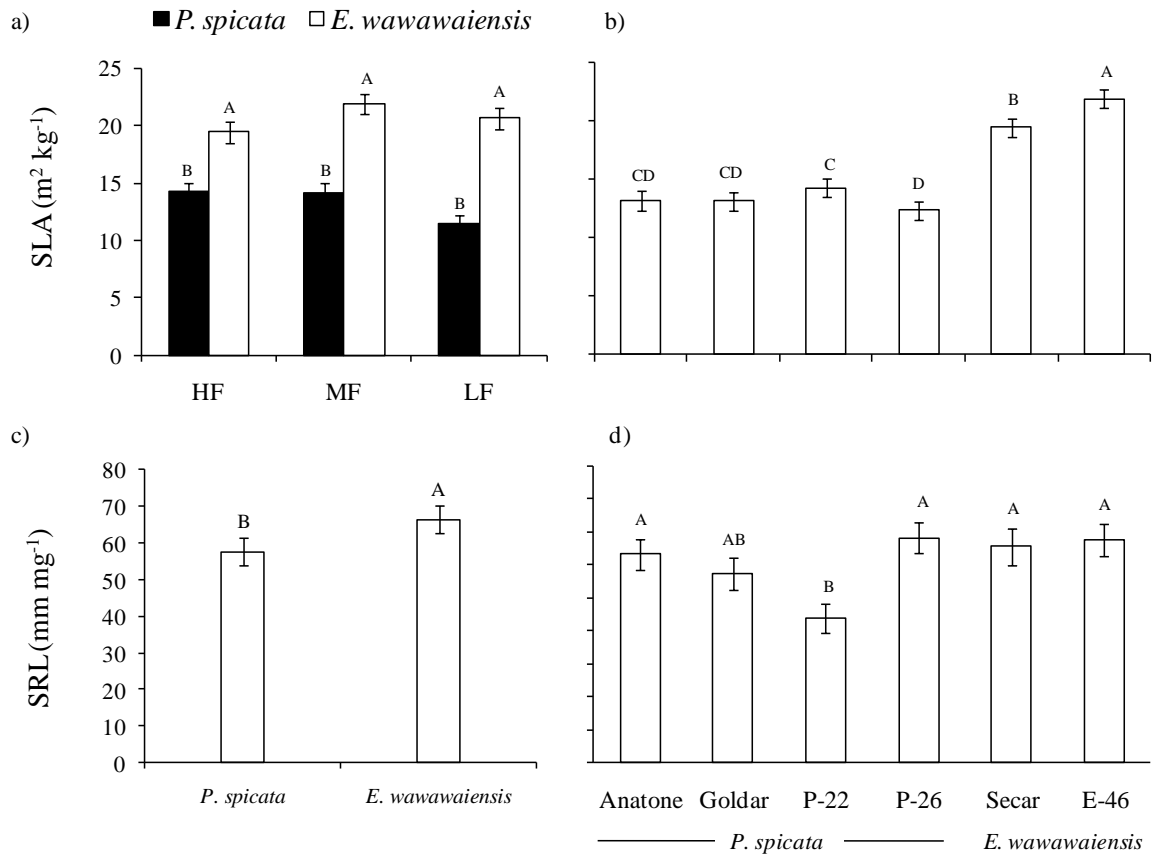


Figure 5. Means and standard errors for (a) specific leaf area (SLA) for *P. spicata* and *E. wawawaiensis* averaged across their respective populations at high (HF), moderate (MF), and low (LF) watering frequencies, (b) SLA for four *P. spicata* and two *E. wawawaiensis* populations averaged across three watering frequencies, (c) specific root length (SRL) for *P. spicata* and *E. wawawaiensis* averaged across their respective populations and three watering frequencies, and (d) SRL of four *P. spicata* and two *E. wawawaiensis* populations averaged across three watering frequencies. Different letters represent significant ($P < 0.05$) differences (a) between the two species within each watering frequency and (b, c, d) between means.

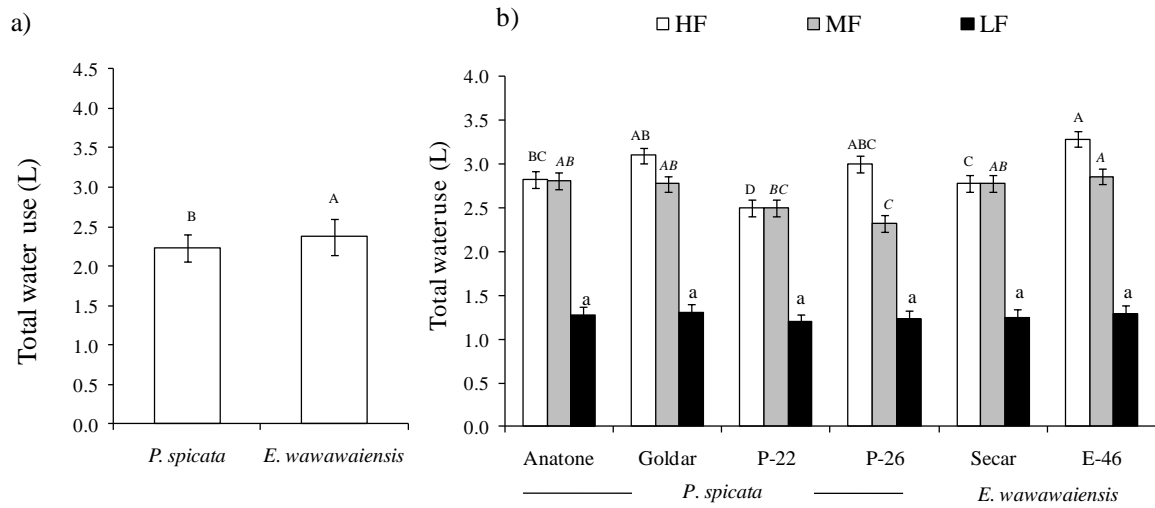


Figure 6. Means and standard errors for total water use by (a) *P. spicata* and *E. wawawaiensis* averaged across their respective populations and three watering frequencies and (b) four *P. spicata* and two *E. wawawaiensis* populations at high (HF), moderate (MF), and low (LF) watering frequencies. Different letters represent significant ($P < 0.05$) differences (a) between species and (b) among populations within high (upper case), medium (upper-case italics), and low (lower case) watering frequencies.

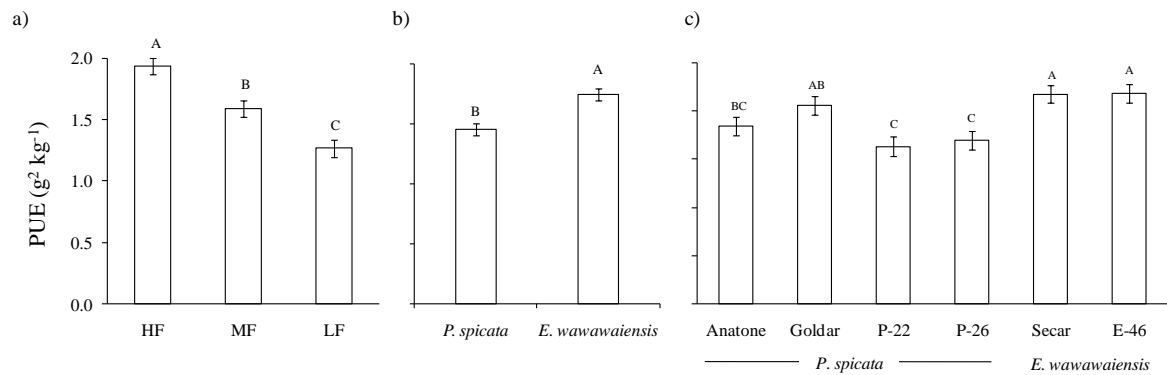


Figure 7. Means and standard errors for precipitation use efficiency (PUE) of (a) high (HF), moderate (MF), and low (LF) watering frequencies averaged across six populations, (b) *P. spicata* and *E. wawawaiensis* averaged across their respective populations and three watering frequencies, and (c) four *P. spicata* and two *E. wawawaiensis* populations averaged across three watering frequencies. Different letters represent significant ($P < 0.05$) differences among means.

CHAPTER 5

COPING WITH HERBIVORY: RELATIVE RESPONSES OF TWO SEMIARID
PERENNIAL BUNCHGRASSES UNDER TWO CLIPPING REGIMES¹**Abstract**

Past overgrazing practices have reduced the abundance of the native bunchgrass, *Pseudoroegneria spicata*, in North America's Intermountain West. This species and *Elymus wawawaiensis*, another Intermountain bunchgrass, are widely used for restoration applications in the region. Defoliation is most detrimental to these grasses at the early-reproductive or 'boot' stage. In practice, however, initial grazing takes place on a common calendar date when livestock are permitted to enter a grazing allotment, at which time individual plants may vary for phenological stage. We compared five *P. spicata* and three *E. wawawaiensis* populations for defoliation response to determine whether tolerance to defoliation, measured as defoliated-to control-shoot biomass ratio, differs when plants are clipped at the same phenological stage ('same-stage') compared to a single common date ('same-date'). Shoot biomass (11%), tiller number (17%), and basal area (12%) were greater for 'same-date' clipping than 'same-stage'. We recorded shoot and root biomass and estimated defoliation tolerance as defoliated-to-control ratio of shoot biomass. However, irrespective of clipping regime, defoliation tolerance of *E. wawawaiensis* (24%) was double that of *P. spicata* (12%), and specific leaf area (SLA) was 27% greater for *E. wawawaiensis*. Populations with greater shoot biomass for the undefoliated control were also less tolerant to defoliation, indicating a trade-off between absolute growth and defoliation tolerance. In contrast, the positive association between SLA and defoliation tolerance suggested there was no trade-off between defoliation

¹ Coauthored by: Jayanti Ray Mukherjee, TA Jones, TA Monaco, and PB Adler

tolerance and relative growth. Thus, in the absence of defoliation, species and populations with lower SLA accumulated more biomass, but following defoliation, those with higher SLA were able to replace shoot biomass faster and better cope with defoliation.

Introduction

Due to low evolutionary grazing pressures, native perennial bunchgrasses of the North American Intermountain West are relatively intolerant of grazing (Mack and Thompson 1982). Since its widespread settlement in the 1840s, grazing by livestock has exerted a dominant influence on the arid-to-semiarid ecosystems of this region (Fleischner 1994) by destabilizing plant communities, increasing the abundance and impacts of invasive plants (Fleischner 1994), reducing native-species abundance (Adler et al. 2005), and reducing livestock productivity (DiTomaso 2000). The lack of grazing tolerance in Intermountain-native bunchgrasses was one factor that motivated the introduction of *Agropyron desertorum* (Fisch. ex Link) Schult., a grazing-tolerant, exotic perennial bunchgrass (Caldwell et al. 1981; Richards and Caldwell. 1985; Monsen et al. 2004). However, because native plants may be preferred by restoration practitioners and livestock grazing continues to be widespread on these rangelands, the development of grazing-tolerant native plant materials is a worthwhile goal.

Grazing tolerance in plants involves confounded interactions between genotype and historical selection pressures (Mack and Thompson 1982; Tiffin and Rausher 1999; Adler et al. 2004); climatic conditions (Milchunas et al. 1988; Adler et al. 2004); and intensity, frequency, and timing of defoliation (DeVal and Crawley 2005). Intensity and frequency of defoliation affects the plant's physiological state, reducing its ability to

compensate for tissue loss (McNaughton 1983; Maschinski and Whitham 1989) and persist in its historical range (Sheley et al. 2008). In arid-to-semiarid environments, severe defoliation nearly always results in undercompensation for tissue loss (Belsky 1986; Maschinski and Whitham 1989). In grasses, rapid recovery in the form of shoot biomass, tiller production, and increased photosynthetic leaf area is often associated with tolerance to defoliation (Caldwell et al. 1981; McNaughton 1983; Belsky et al. 1993; Guo et al. 2008).

Specific leaf area (leaf area per biomass; SLA) is the best single predictor of relative growth rate (RGR) (Reich et al. 2003; Poorter and Garnier 2007) and may be used as a surrogate for RGR to understand variation among species for regrowth capacity. Research has shown that defoliation may increase (Guo et al. 2008) or have no effect (Rotundo and Aguiar 2008; Thorne and Frank 2009) on SLA. The plant ecophysiology literature recognizes an evolutionary tradeoff between a genotype's ability to grow rapidly and to tolerate resource shortages (Chapin 1980; Grime 2001). Similarly, when there is reduced biomass following defoliation, yet resources remain unchanged, less productive species or populations will be more defoliation tolerant than more productive ones.

Timing of defoliation strongly influences plants' ability to recover (McNaughton 1985; Maschinski and Whitham 1989). In cool-season grasses, defoliation at the early-reproductive phase, i.e., the 'boot' stage, is more detrimental than defoliation at vegetative or late-reproductive stages (Blaisdell and Pechenec 1949; Cook and Stoddart 1963; Wilson et al. 1966; Ganskopp 1988; Sheley et al. 1997; Jones and Nielson 1997). The plant is at the boot stage when the culm is swollen with the inflorescence prior to its

emergence above the flag leaf (Wilson et al. 1966). When culm is elongated, apical meristem and culm leaves are elevated and more susceptible to defoliation and without replacement of apical or intercalary meristems, tissue replacement becomes a slow process (Caldwell et al. 1981). However, in all except the last of the six above-mentioned studies, mature grasses were compared by defoliating on a single common date regardless of phenological stage. However, defoliation on a common date, relative to defoliation at the boot stage, may lead to an inflated estimate of defoliation tolerance. This is because the impact on plants, populations, or species not at the boot stage is expected to be less severe, causing a bias in estimation of defoliation tolerance

Pseudoroegneria spicata (Pursh.) A. Löve, is a widely occurring, native, C₃ perennial bunchgrass that was once dominant in the Intermountain West (Mack and Thompson 1982; Monsen et al. 2004). Poor grazing tolerance, coupled with high palatability, is believed to be responsible for the decline of this species (Jones and Nielson 1997; Adler et al. 2004). This grass is grazing sensitive and recovers slowly from severe defoliation (Mueggler 1975; Caldwell et al. 1981; Richards and Caldwell 1985; Jones and Nielson 1997). Newly generated photosynthetic area is believed to be important for *P. spicata*'s recovery (Richards and Caldwell 1985). However, recovery is compromised by prolonged investments in root growth, at the expense of shoot growth, even after severe defoliation (Caldwell et al. 1981). Despite its sensitivity to defoliation, however, *P. spicata* is widely used in rangeland restoration efforts in the region.

Elymus wawawaiensis J. Carlson & Barkworth, another native, C₃ perennial bunchgrass with a more limited range than *P. spicata*, has been reported to be more defoliation tolerant than *P. spicata* (Jones and Nielson 1997). Owing to a superficial

morphological resemblance, these two grasses were taxonomically confused until 1986 (Carlson and Barkworth 1997). Since the release of ‘Secar’ (now known to be *E. wawawaiensis*) in 1980, this plant material has become known for its superior productivity, seedling establishment, and drought tolerance relative to most commercially available *P. spicata* plant materials (Morrison and Kelley 1981; Jones and Nielson 1997; Ogle 2002; Monsen et al. 2004). Thus, *E. wawawaiensis* has continued to be widely used as a surrogate for *P. spicata* in rangeland restoration.

In this study, we applied spring defoliation for two consecutive years under two clipping regimes (‘same-stage’ and ‘same-date’) to five populations of *P. spicata* and three populations of *E. wawawaiensis*, along with non-spring-defoliated controls. Our first objective was to identify superior defoliation-tolerant species and populations within species among those chosen for examination here because of their commercial importance or experimental interest. Our second objective was to compare ‘same-stage’ versus ‘same-date’ clipping regimes for overall severity of defoliation and the relative defoliation tolerance of species and populations. Our third objective was to identify traits that might be associated with defoliation tolerance, e.g., shoot biomass, SLA, leaf length, tiller number, and plant basal area, and to elucidate the relationships between these traits and defoliation tolerance.

Based on the above-mentioned studies, we expected that, while both *P. spicata* and *E. wawawaiensis* would respond to defoliation by undercompensation, two consecutive years of defoliation would have a greater negative impact on *P. spicata* than on *E. wawawaiensis* in terms of shoot biomass, tiller number, plant-basal area, and leaf length. Secondly, we predicted that the impact of defoliation at the boot stage (‘same-

stage'), when averaged across populations, would be more severe than defoliation on a common date and that clipping regime would interact with species and populations for defoliation tolerance. Thirdly, we anticipated that defoliation would increase SLA, and species and populations with higher SLA would display greater defoliation tolerance. We also predicted a negative correlation between defoliation tolerance and undefoliated-control shoot biomass, and we expected that more defoliation-tolerant populations would display greater regrowth tiller number, greater plant basal area, and longer leaves.

Materials and methods

For this study, we used two released commercial *P. spicata* populations ('Goldar' and Anatone germplasm), three experimental *P. spicata* populations (P-22, P-26, and P-27), two released commercial *E. wawawaiensis* populations ('Secar' and 'Discovery') and one experimental *E. wawawaiensis* population (E-46). Of the *P. spicata* experimental populations, P-22 was developed from P-1, which is of unknown origin, by four cycles of selection (one of which was for recovery after clipping). P-26 was developed by three cycles of selection for seedling vigor and spike production (no emphasis on clipping tolerance) from the commercially released P-7 germplasm, a genetically diverse polycross developed by intermating 25 populations. P-27, the lone tetraploid *P. spicata* population in the study, was developed by two cycles of selection for seedling vigor and spike production on P-8. P-8, in turn, was experimentally induced from the diploid P-3, a polycross of several populations collected along the Grande Ronde River in northeastern Oregon. E-46 *E. wawawaiensis* was developed from Discovery *E. wawawaiensis* by one cycle of selection for clipping tolerance and another cycle for seedling vigor and spike production.

Seeds of these populations were germinated in plastic boxes at room temperature, and after a week, seedlings were transplanted into a 3:1 sand:peat moss mixture in opaque, round plastic tubes (20-cm long and 5-cm wide) in the USDA-ARS greenhouse on the Utah State University campus in Logan, Utah. Plants were trimmed to a 15-cm height before transplanting to the field site. Plants of eight populations were planted in pairs in a randomized 16-by-16 square arrangement (1-m spacing in both directions) on 5 June 2006 at Millville, Utah. Soil at the Millville site is a Ricks gravelly loam (coarse-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Calcic Haploxerolls). An equidistant one-plant border was planted around the perimeter of the plot area. There were 30 squares of this 16-plant arrangement, each of which served as a replicate. Throughout the experiment, clippings were applied at a 10-cm height.

We randomly selected one individual within each pair for the defoliation treatment, and the other individual was designated as the undefoliated control. We randomly selected 15 of the 30 replicates for the ‘same-stage’ clipping regime, while the remaining were designated for the ‘same-date’ clipping regime. For ‘same-stage’, plants were initially defoliated at the boot stage, and subsequent clippings were made at 4-week intervals (Table 1a, b). For ‘same date’, plants were initially defoliated on a common calendar date when two-thirds of the plants of Secar *E. wawawaiensis* reached head emergence (Table 1a, b) (Jones and Nielson 1997) (see results for details) in 2007 and 2008.

Thus, a total of 480 plants across two clipping regimes (‘same-stage’ and ‘same-date’), five populations of *P. spicata* and three populations of *E. wawawaiensis*, and two defoliation treatments (spring-defoliated and non-spring-defoliated control) were

arranged in a split-split plot design. Clipping regime was the whole-plot factor, and 15 replicates were nested within each clipping regime. Populations were the split-plot factor, and defoliation treatment was the split-split-plot factor. No defoliation treatments were applied in 2006, the establishment year, but annual biomass production was harvested on 29 October (Table 1a). In 2007, between 7 and 16 May, we harvested biomass from the plants assigned to the 'same-stage' clipping regime (Table 1b). We harvested biomass from the 'same-date' plants on 21 May, when most individuals of Goldar, Anatone, and P-26 had already completely headed (Table 1a). For defoliated plants, we clipped three additional times at four-week intervals from the initial spring defoliation. For plants designated as undefoliated controls, total annual biomass was not removed again until 13 August 2007. For both clipping regimes and both defoliation treatments, we harvested fall regrowth on 10 October. Hence, for 2007, plants were subjected to a total of two harvests for the control treatment and five for the spring-defoliation treatment.

On 3-4 May 2008, a subsample of five leaves from each plant was passed through a LI-3100C leaf-area meter (LI-COR leaf-area meter, Lincoln, Nebraska), after which leaves were dried (60°C for 2 days), weighed, and SLA was determined. We took the initial harvests for the 'same-stage' clipping regime between 24 May and 19 June (Table 1b) and for the 'same-date' clipping regime on 16 June (Table 1a). In 2008, shoot biomass regrowth was reduced relative to 2007 due to drought (See the Appendix), therefore we defoliated plants only two additional times during the summer, again at four-week intervals. We counted tillers on control and defoliation treatments shortly before the initial spring harvest 15 May 2008. On 12 August, the final summer biomass was harvested from both control and defoliated plants. Following two consecutive years

of defoliation, on 3 and 4 October, SLA was determined on a subsample of five green leaves without necrosis from each plant. At the same time, leaf length was measured on the same subsample using WinRHIZO Pro Version 2005b (Reagent Instrument Inc., Québec City, Canada), and basal area was measured on individual plants. Finally, on 20 October, we harvested fall regrowth shoot biomass. Thus, for 2008, plants were subjected to a total of two harvests for the control and four harvests for the spring-defoliation treatment.

Defoliated-to-control shoot-biomass ratio may be used to estimate defoliation tolerance (Jones and Nielson 1997). For 2008 and 2009, we estimated percentage defoliation tolerance as the ratio of the dry shoot biomass of the spring-defoliated member of each pair to the dry shoot biomass of its corresponding control plant x 100. Data were analyzed using SAS 9.1.3 (SAS Institute, 2003). All effects were considered fixed except replication, which was nested within clipping regime. Data for shoot biomass and SLA were analyzed as repeated measures in PROC MIXED with year as a fixed factor. Tiller number, tiller mass, and basal area data were also analyzed with PROC MIXED, but these data were not repeated. When four-way and three-way interactions were not significant, they were removed from the model, but in these cases, statistical significance of the remaining interactions did not change. Data were transformed when normality assumptions were not met. A square-root transformation was used for shoot biomass, and an inverse-square root transformation was used for SLA and defoliation tolerance. We compared species and years using CONTRAST statements. We separated least-squares means with an LSD at $P < 0.05$. We plotted back-transformed

means, and mean separations were generated from the transformed data. However, standard errors were calculated from the untransformed data set.

Results

Species did not vary for shoot biomass or tiller number in the establishment year (2006), but populations within species varied for both traits (Table 2). Within *P. spicata*, P-22 exhibited the greatest shoot biomass, while P-27 exhibited the least, and within *E. wawawaiensis*, E-46 and Discovery displayed greater shoot biomass than Secar (Fig. 1a). P-27 produced only about one-third the number of tillers of the other *P. spicata* populations, while *E. wawawaiensis* populations were similar for tiller number (Fig. 1b). In 2006, *E. wawawaiensis* exhibited 38% greater SLA than *P. spicata* (Table 2, Fig. 1c). Goldar and P-22 had the highest SLA among *P. spicata* populations, with P-26 and P-27 being lowest, while E-46 *E. wawawaiensis* had greater SLA than Secar and Discovery.

Average heading date was 26 days earlier in 2007 than 2008 ($P < 0.0001$). On average, *P. spicata* populations headed three days earlier than *E. wawawaiensis* populations ($P < 0.01$). Among *P. spicata* populations, Anatone, P-22, and P-26 exhibited earlier head emergence than Goldar and P-27. Goldar was the latest *P. spicata* population in 2007 (Fig. 2), while in 2008, P-27 headed four to seven days later than the other *P. spicata* populations. Compared to the *P. spicata* populations, the *E. wawawaiensis* populations were less variable for heading date.

On average, the ‘same-date’ clipping regime yielded about 11% greater shoot biomass than ‘same-stage’ ($P < 0.05$). However, interactions involving clipping regime and species or populations within species were not significant ($P > 0.10$) (Table 3). In the first year of defoliation (2007), defoliation reduced shoot biomass 21%, but the effect

increased to 83% in 2008 and 77% in 2009. For the control, the two species were similar in 2007 and 2009, while in 2008 control plants of *P. spicata* were 31% more productive than those of *E. wawawaiensis* (Fig. 3). Under defoliation, shoot biomass was similar for the two species in 2007 and 2008, while in 2009 defoliated *E. wawawaiensis* was 121% more productive than defoliated *P. spicata* (Fig. 3). P-27 exhibited the greatest increase (73%) in control shoot biomass from 2007 to the following years (Fig. 4a) and the greatest decrease (87%) in defoliated-shoot biomass across the same time span (Fig. 4b). This population was responsible for generating the significant ($P < 0.01$) year x defoliation x population within species interaction for shoot biomass.

On average, *E. wawawaiensis* populations (24%) displayed twice the defoliation tolerance of *P. spicata* populations (12%) ($P < 0.0001$). P-26 *P. spicata* was more than twice as defoliation tolerant at 'same-date' than at 'same-stage', while P-27 was nearly three-fourths less tolerant at 'same-date' compared to 'same-stage', generating a significant interaction between clipping regime and *P. spicata* populations ($P < 0.05$) (Fig. 5a). A significant interaction of year and populations within species ($P < 0.005$) occurred because Goldar was 66% less defoliation tolerant in 2009 than in 2008, as opposed to other *P. spicata* populations, which exhibited higher defoliation tolerance in 2009 (Fig. 5b). Defoliation tolerance of *Elymus wawawaiensis* populations did not interact with clipping regime or year (Fig. 5a, b).

Clipping regime and its interactions did not affect SLA (Table 3; Fig. 5). On average, SLA of *E. wawawaiensis* was 27% greater than *P. spicata* ($P < 0.0001$). The *E. wawawaiensis* populations were similar for SLA, but of the *P. spicata* populations, Anatone and Goldar displayed the greatest SLA, while P-27 demonstrated the least. The

effect of defoliation on SLA differed between May and October 2008. This interaction was weakly significant ($P < 0.10$) because defoliation reduced SLA of *P. spicata* 14% relative to the control in May, but not in October. Defoliation, on the other hand, did not affect the SLA of *E. wawawaiensis* for either May or October. The two species and all populations showed significantly ($P < 0.0001$) greater SLA in October relative to May. Anatone *P. spicata* (107%) and Secar *E. wawawaiensis* (99%) showed the greatest increases in SLA in October relative to May (Fig. 5c).

Similar to shoot biomass, plant basal area (measured in August 2008) was 12% greater for 'same-date' than 'same-stage' ($P < 0.05$), but clipping regime did not interact with species or populations (Table 4). The two species had similar plant basal area in the control treatment, but defoliation reduced plant basal area 34% for *E. wawawaiensis* and 47% for *P. spicata*, generating a defoliation x species interaction ($P < 0.01$). Among *E. wawawaiensis* populations, E-46 had 18% greater plant basal area than Secar, but we detected no differences among *E. wawawaiensis* populations for reduction in plant basal area from spring defoliation ($P > 0.10$). Among *P. spicata* populations, not only did P-27 display the lowest control plant basal area (Fig. 6a), it also displayed the greatest reduction in plant basal area upon defoliation (65%).

After one year of spring defoliation (May 2008), tiller number varied neither with clipping regime nor species (Table 4). We did find differences among *P. spicata* populations, with P-27 displaying fewest tillers. On the other hand, *E. wawawaiensis* populations were similar for tiller number. Overall, spring defoliation decreased May 2008 tiller number by 49% ($P < 0.0001$), but defoliation responses differed among species and PSSP populations (Table 4). Tiller number of *P. spicata* populations was

33% greater than *E. wawawaiensis* for the control, while under spring defoliation, *E. wawawaiensis* populations displayed 26% more tillers than *P. spicata*. However, P-27 *P. spicata* accounted for a disproportionate amount of this species' reduction in tiller number in response to defoliation (Fig. 6b). In contrast to *P. spicata*, the negative impact of defoliation on tiller number was similar across *E. wawawaiensis* populations.

For the spring-defoliation treatment, tiller number varied with clipping regime ($P < 0.05$), with 'same-date' exhibiting 17% greater tiller number than 'same-stage'. While the two species did not differ ($P > 0.05$) in tiller number, *P. spicata* populations did differ ($P < 0.0001$) for this trait. This was primarily because P-27 exhibited, on average, 60% fewer tillers than other *P. spicata* populations. Clipping regime also interacted with month and year ($P < 0.0001$) for tiller number (Fig. 7a), and this interaction was again due to P-27 *P. spicata* (Fig. 7b).

Leaf length was not influenced by clipping regime ($P > 0.10$) (Table 4). On average, *E. wawawaiensis* displayed 13% greater leaf length than *P. spicata* ($P < 0.0001$). While *E. wawawaiensis* accessions were similar in leaf length, P-27 and P-26 (both 22.0 ± 0.7 cm) *P. spicata* had significantly longer leaves than Anatone (20.2 ± 0.7 cm) and Goldar (20.0 ± 0.7 cm). Spring defoliation reduced leaf length 7% ($P < 0.01$), but species and populations within species did not interact with defoliation ($P > 0.10$).

We found a significant negative correlation ($r = -0.81$, $P < 0.05$) between shoot biomass and SLA for the undefoliated control (Fig. 8a), while this relationship was positive ($r = 0.83$, $P < 0.05$) for the spring-defoliation treatment (Fig. 8b). Plant basal area was not correlated with shoot biomass ($r = 0.44$, $P > 0.05$) or SLA ($r = -0.33$, $P < 0.005$) for the control, but plant basal area displayed a positive correlation with shoot biomass (r

= 0.81, $P < 0.05$) and SLA ($r = 0.87$, $P < 0.005$) under spring defoliation, similar to the results for shoot biomass. We found a negative correlation between control shoot biomass and defoliation tolerance ($r = -0.75$, $P < 0.05$) for ‘same stage’ (Fig. 8c), while the same was not significant ($P > 0.05$) for ‘same-date’ (Fig. 8d). On the other hand, a positive correlation between SLA and defoliation tolerance ($r = 0.80$, $P < 0.05$) was noted for ‘same stage’ (Fig. 8e), while for ‘same-date’, SLA and defoliation tolerance were not related (Fig. 8f).

Discussion

Intense defoliation stimulates overcompensation in humid systems (van der Heyden and Stock 1996; Ruiz et al. 2008), while limited moisture availability in arid and semiarid environments impedes growth, resulting in undercompensation (Belsky 1986; Ruiz et al. 2008). In relation to our first objective, both species responded to defoliation by undercompensation, and defoliation for two consecutive years reduced total biomass 71% for *P. spicata* and 58% for *E. wawawaiensis*. The two species had similar defoliated shoot biomass in 2007 and 2008, yet on average, *E. wawawaiensis* was twice as defoliation tolerant as *P. spicata*. This was because *E. wawawaiensis* showed significant improvement in shoot biomass production in 2009, when spring precipitation returned to normal levels after two years of low spring precipitation (See the Appendix). Discovery and E-46 *E. wawawaiensis* populations were primarily responsible for this improvement in shoot biomass in the final year. Populations within both species were similarly tolerant to defoliation, with the exception of P-27 *P. spicata*.

For our second objective, we wanted to determine whether relative defoliation tolerance of species and populations varied with clipping regime. In accordance with our

prediction, plants subjected to the ‘same-date’ clipping regime generally displayed greater productivity and plant basal area than ‘same-stage’ plants. Evidently, clipping individual plants at the same phenological stage generated a more uniform and generally more severe response than clipping on a single date. Thus, our results suggest that, for selection of defoliation tolerance, it is more appropriate to apply defoliation at a common phenological stage, preferably the boot stage, than a common date. Also supporting this argument was the finding that correlations between defoliation tolerance and both shoot biomass and SLA were significant only for the ‘same-stage’ regime.

We expected to find an interaction between clipping regime and species and populations for defoliation tolerance. While we detected an interaction for *P. spicata* populations, no such interaction was found for *E. wawawaiensis* populations. Two populations, P-26 and P-27, were particularly responsible for the *P. spicata* interaction. P-26 was the most defoliation tolerant of all *P. spicata* populations under ‘same-date’, while Anatone was higher than P-26 under ‘same-stage’. The reason for the especially high defoliation tolerance of P-26 under ‘same-date’ is unclear, as its phenological dates were similar to most other *P. spicata* populations. In contrast to P-26, the least defoliation-tolerant population, P-27, displayed 73% less defoliation tolerance under the ‘same-date’ regime than ‘same-stage’, while other *P. spicata* and *E. wawawaiensis* populations displayed higher tolerance under ‘same-date’. This might be explained by the later maturity of this population in 2008, as ‘same-date’ defoliation was performed when P-27 was much closer to the boot stage than the other populations, which would have been much later in phenology at this time.

For our third objective, we wished to identify traits associated with defoliation tolerance and to describe these biological relationships. Populations of *E. wawawaiensis*, the more defoliation-tolerant species, displayed greater leaf length and SLA than *P. spicata* populations. Thus, consistent with our prediction, the species with greater SLA was also the more defoliation tolerant. High SLA is often associated with a grazing-tolerance strategy, not only because it permits higher growth potential following defoliation (McNaughton 1984), but also because less biomass investment per unit leaf area reduces the cost of defoliation (Rotundo and Aguiar 2008).

Our study did not provide any evidence that SLA of regrowth was higher than SLA for leaves of control plants. Rather, in contrast to our prediction, SLA for defoliated plants was less than that of controls. This was primarily because SLA of *P. spicata* was reduced in May 2008 following 2007 defoliation, while *E. wawawaiensis* remained unaffected, evidenced by a weak defoliation x time x population interaction. This might be an indication that *P. spicata* reduces leaf area in response to defoliation to a greater extent than *E. wawawaiensis*. However, October 2008 SLA did not differ between control and defoliation treatments. Therefore, our finding was partially consistent with other greenhouse studies on grasses in which SLA did not differ between control and defoliated plants (Rotundo and Aguiar 2008; Thorne and Frank 2009). We also observed a significant increase in SLA between May and October. This increase is possibly due to two confounding effects: first, reduced biomass accumulation during the end of the growing season while leaf area remained unchanged or second, increased leaf area, possibly stimulated by the August defoliation, leading to greater SLA for both treatments. But whatever the reason, the increase for SLA between May and October did not vary

among species or populations. Despite the innate ability of high SLA to promote growth, severe defoliation reduced SLA (May 2008), which might not have occurred at milder defoliation levels (Ruiz et al. 2008). Species with high RGR have been shown to produce greater seedling biomass, while low-RGR species accumulate more biomass over time (Reich et al. 2003). As SLA is known to be the best predictor for RGR (Poorter and Garnier 2007), this may explain why we found that the relationship between shoot biomass and SLA was negative for the control (mostly mature growth), yet positive for the defoliation treatment (mostly immature growth).

Plant ecophysiological studies suggest a trade-off between growth and tolerance (Grime 1977; Chapin 1980; Grace 1991). Hence, we expected that there would be a negative correlation between defoliation tolerance and control shoot biomass. Consistent with our prediction, under the ‘same-stage’ regime we found that shoot biomass of the undefoliated control was negatively correlated with defoliation tolerance, confirming a trade-off between tolerance and growth (Grime 1977; Chapin 1980; Grime 2001). However, we also found a positive correlation between defoliation tolerance and SLA, the best predictor of RGR (Poorter and Garnier 2007), indicating a lack of a tradeoff between tolerance and growth between the two species. This seeming conflict may be resolved if defoliation tolerance is compromised by high absolute growth, as for *P. spicata*, yet enhanced by high relative growth, as for *E. wawawaiensis*. Thus, species with lower SLA may be more productive over longer periods of time, but following defoliation, the species with higher SLA may be able to generate new tissues faster. The repeated-measures analysis on tiller number of defoliated plants showed that tiller number per plant declined substantially after spring defoliation in both years. *Agropyron*

desertorum, an exotic bunchgrass introduced to the Intermountain West, is considered to be more defoliation tolerant than *P. spicata* (Caldwell et al. 1981), and this has been attributed to the former's rapid production of new tillers with three to five times photosynthetic surface area (Caldwell et al. 1981). Tillering is a complex physiological procedure (Murphy and Briske 1992). In addition to the release of the inhibitory effect of apical meristem, extreme spatial and temporal resource availability and environmental variability of rangeland conditions may stimulate axillary-bud growth (Murphy and Briske 1992). In our study, with the exception of P-27, defoliated plants of *P. spicata* exhibited greater tiller number than defoliated plants of *E. wawawaiensis*. This implies that *E. wawawaiensis*' greater defoliation tolerance is likely due to greater surface area per tiller rather than to greater tiller number. Apparently, the superior defoliation tolerance of *E. wawawaiensis* relative to *P. spicata* operates by a different mechanism than does *A. desertorum*.

Plant basal area is consistently associated with plant biomass and vigor (Sala et al. 1986), and defoliation decreases plant basal area of bunchgrasses (Butler and Briske 1988). Across populations, we found that plant basal area was highly positively correlated with shoot biomass and SLA for defoliated plants but not for the control. We expected that a more defoliation-tolerant population would have greater plant basal area, and consistent with our prediction, plant basal area was greater for *E. wawawaiensis* than *P. spicata*. High plant basal area was particularly noted for Discovery and E-46, the two populations that showed a significant increase in defoliated-shoot biomass in 2009. However, it is important to mention that we observed fragmentation of the original large tussock into multiple smaller ones for defoliated plants of Discovery (23%) and E-46

(10%) *E. wawawaiensis*, as well as P-26 (3%) *P. spicata*, while fragmentation was not evident in other populations, a trend also noticed in other bunchgrasses (Butler and Briske 1988).

Consistent with our predictions, *E. wawawaiensis* was less severely affected by defoliation than *P. spicata* in terms of shoot biomass, SLA, leaf length, and plant basal area. Conversely, *P. spicata* (except P-27) produced more tillers than *E. wawawaiensis* and was more productive for undefoliated-control biomass. We conclude that superior defoliation tolerance of *E. wawawaiensis* may be attributed to its proportionately greater photosynthetic leaf area compared to *P. spicata*, while tiller recovery seems to contribute more to *P. spicata*'s tolerance. P-27's defoliation response, which was especially poor compared to the other *P. spicata* populations, demonstrates the wide range of this species' genotypic variation. Finally, we detected a trade-off between absolute growth rate and defoliation tolerance, while relative growth rate was positively associated with defoliation tolerance, suggesting that rapid replacement of leaf area and reestablishment of the pre-clipping canopy (Caldwell et al. 1981) is an evolutionary response to cope with herbivory (Belsky 1986). Our results showed that two consecutive years of defoliation caused significant damage to both species. Hence, for restoration purposes, consecutive years of grazing will likely be detrimental to these species, as reported by Brewer et al. (2007). Alternate-year grazing would be more desirable for both species, allotting greater time for their recovery. Seasonal distribution of grazing in the pastures, by turning in cattle after the critical stage in the life cycle of native grasses (Frandsen 1950; Merrill 1954), may be a desirable alternative to help retain plant productivity and improve rangeland conditions. Our study also suggests that, due to its faster recovery from

defoliation, *E. wawawaiensis* may be preferred over *P. spicata* in restoration seed mixes for Intermountain rangelands when they are utilized for livestock grazing.

References

- Adler PB, Milchunas DG, Lauenroth WK, Sala OE, Burke IC (2004). Functional traits of graminoids in semi-arid steppes: a test of grazing histories. *J App Ecol* 41: 653–663
- Adler PB, Milchunas DG, Sala OE, Burke IC, Lauenroth WK (2005) Plant traits and ecosystems grazing effects: comparison of U.S. sagebrush steppe and Patagonia steppe. *Ecol Appl* 15: 774-792. DOI: 10.1890/04-0231
- Belsky AJ (1986) Does herbivory benefit plants? A review of the evidence. *Am Nat* 127: 870–892
- Belsky JA, Carson WP, Jensen CL, Fox JA (1993) Overcompensation by plants: herbivore optimization or red herring? *Evol Ecol* 7: 109-121
- Blaisdell JP, Pechanec JF (1949) Effects of herbage removal at various dates on vigor of bluebunch wheatgrass and arrowleaf balsamroot. *Ecology* 30: 298-305
- Brewer TK, Mosley JC, Lucas DE, Schmidt LR (2007) Bluebunch wheatgrass response to spring defoliation on foothill rangeland. *Rangeland Ecol Manage* 60: 498-507
- Butler JL, Briske DD (1998) Population structure and tiller demography of the bunchgrass *Schizachyrium scoparium* in response to herbivory. *Oikos* 51: 306-312
- Caldwell MM, Richards JH, Johnson DA, Nowak RS, Dzurec RS (1981) Coping with herbivory: Photosynthetic capacity and resource allocation in two semi-arid *Agropyron* bunchgrasses. *Oecologia* 50: 14–24

- Carlson JR, Barkworth ME (1997) *Elymus wawawaiensis*: a species hitherto confused with *Pseudoroegneria spicata* (Triticeae, Poaceae). *Phytologia* 83:312-330
- Chapin FS (1980) The mineral nutrition of wild plants. *Ann Rev Ecol Syst* 11: 233-260
- Cook CW, Stoddart LA (1963) The effect of intensity and season of use on the vigor of desert range plants. *J Range Manage* 16: 315-317
- Del-Val E, Crawley MJ (2005) What limits herb biomass in grasslands: competition or herbivory? *Oecologia* 142: 202–211. DOI: 10.1007/s00442-004-1719-8
- DiTomaso JM (2000) Invasive weeds in rangelands: species, impacts, and management. *Weed Sci* 48: 255-265
- Fleischner TL (1994) Ecological costs of livestock grazing in western North America. *Conserv Biol* 8: 629-244
- Frandsen WR (1950) Management of reseeded ranges. *J Range Manage* 3: 125-129
- Ganskopp D (1988) Defoliation of Thurber needlegrass: herbage and root responses. *J Range Manage* 41: 472-476
- Grace JB (1991) A clarification of the debate between Grime and Tilman. *Funct Ecol* 5: 583-587
- Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am Nat* 111: 1169-1194
- Grime JP (2001) Plant strategies, vegetation processes, and ecosystems properties (2nd ed.). John Wiley & Sons Ltd., Chichester, UK
- Guo Y, Wang D, Ba L, Bai Y, and Liu B (2008) Interactions between herbivory and resource availability on grazing tolerance of *Leymus chinensis*. *J Exp Bot* 63: 113-122. DOI:10.1016/j.envexpbot.2007.10.030

- Jones TA, Nielson DC (1997) Defoliation tolerance of bluebunch and Snake River wheatgrasses. *Agron J* 89: 270–275
- Mack RN, Thompson JN (1982) Evolution in steppe with few large, hooved mammals. *Am Nat* 119: 757–773
- Maschinski J, Whitham T (1989) The continuum of plant responses to herbivory, the influence of plant association, nutrient availability, and timing. *Am Nat* 134: 1-19
- McNaughton SJ (1983). Compensatory plant growth as a response to herbivory. *Oikos* 40: 329–336
- McNaughton SJ (1984) Grazing lawns: animals in herds, plant form, and coevolution. *Am Nat* 124: 863-886. DOI: 10.1086/284321
- McNaughton SJ (1985) Ecology of a grazing ecosystem: the Serengeti. *Ecol Monog* 55: 259-294. DOI: 10.2307/1942578
- Merrill LB (1954) A variation of deferred rotation grazing for use under southwest range conditions. *J Range Manage* 7: 152-154
- Milchunas DG, Sala OE, Lauenroth WK (1988) A generalized model of the effects of grazing by large herbivores on grassland community structure. *Am Nat* 132: 87–106
- Monsen SB, Stevens R, Shaw NL (2004) Restoring western ranges and wildlands. U.S. Forest Service Gen. Tech. Rep. RMRS-GTR 136 (Vol. 2). Fort Collins, Colorado, USA
- Morrison KJ, Kelley CA (1981) Secar bluebunch wheatgrass. EB 0991. Washington State University, Cooperative Extension, Pullman, Washington, USA

- Mueggler WF (1975). Rate and pattern of vigor recovery in Idaho fescue and bluebunch wheatgrass. *J Range Manage* 28: 198–204
- Murphy JS, Briske DD (1992) Regulation of tillering by apical dominance: chronology, interpretive value, and current perspectives. *J Range Manage* 45: 419-429
- Ogle D (2002) Plant fact sheet: Snake River wheatgrass. Available via DIALOG. http://plants.usda.gov/factsheet/pdf/fs_elwa2.pdf . Accessed 15 Oct 2009
- Poorter H, Garnier E (2007) Ecological significance of inherent variation in relative growth rate and its components. In: Pugnaire FI, Valladares F (eds) *Functional Plant Ecology*. CRC Press, Boca Raton, Florida, USA, pp 67–100
- Reich PB, Wright IJ, Cavender-Bares J, Craine JM, Oleksyn J, Westoby M, Walters MB (2003) The evolution of plant functional variation: traits, spectra, and strategy. *Int J Plant Sci* 164: S143-S164. DOI: 10.1086/374368
- Richards JH, Caldwell MM (1985) Soluble carbohydrates, concurrent photosynthesis and efficiency in regrowth following defoliation: a field study with *Agropyron* species. *J App Ecol* 22: 904-920
- Rotundo JL, Aguiar MR (2008) Herbivory resistance traits in populations of *Poa ligularis* subjected to historically different sheep grazing pressure in Patagonia. *Plant Ecol* 194: 121-133. DOI 10.1007/s11258-007-9279-8
- Ruiz –R N, Ward D, Saltz D (2008) Leaf compensatory growth as a tolerance strategy to resist herbivory in *Pancratium sickenbergeri*. *Plant Ecol* 198: 19–26. DOI 10.1007/s11258-007-9381-y

- Sala OE, Osterheld M, Leon RJC, Soriano A, Osterheldt M (1986) Grazing effects on plant community structure in subhumid grasslands of Argentina. *Vegetatio* 67:27-32
- SAS (2003) SAS/STAT user's guide, SAS Institute version 9.1 Cary, North Carolina, USA
- Sheley RL, Bingham BS, Svejcar TJ (2008) Crested wheatgrass defoliation intensity and season on medusahead invasion. *Rangeland Ecol Manage* 61: 211-217. DOI: 10.2111/07-062.1
- Sheley RL, Olson BE, Larson LL (1997) Effect of weed seed rate and grass defoliation level on diffused knapweed. *J Range Manage* 50: 39-43
- Thorne MA, Frank DA (2009) The effects of clipping and soil moisture on leaf and root morphology and root respiration in two temperate and two tropical grasses. *Plant Ecol* 200: 205-215. DOI 10.1007/s11258-008-9445-7
- Tiffin P, Rausher MD (1999) Genetic constraints and selection acting on tolerance to herbivory in the common morning glory *Ipomea purpurea*. *Am Nat* 154: 700-716
- van der Hayden F, Stock WD (1996) Regrowth of a semiarid shrub following simulated browsing: the role of reserve carbon. *Funct Ecol* 10: 647-653
- Wilson AM, Harris GA, Gates DH (1966) Cumulative effects of clipping on yield of bluebunch wheatgrass. *J Range Manage* 19: 90-91

Table 1. (a) Clipping schedule for “same-date” and “same-stage” clipping regime and undefoliated control in 2006, 2007, 2008, and 2009 and (b) mean, minimum, and maximum, dates of heading for the ‘same stage’ and ‘same date’ clipping regimes for five *P. spicata* and three *E. wawawaiensis* populations for the first (2007) and second (2008) years of defoliation.

(a)	Clipping schedule	‘Same-stage’ clipping regime **	‘Same-date’ clipping regime	Undefoliated control
	2006	29 October ¹	29 October ¹	29 October ¹
	2007 (1 st clipping)	7 May - 16 May	21 May	---
	2007 (2 nd clipping)	4 June - 13 June	18 June	---
	2007 (3 rd clipping)	2 July - 11 July	16 July	---
	2007 (4 th clipping)	30 July - 8 August	13 August	13 August ¹
	2007 (5 th clipping)	10 October	10 October	10 October ¹
	2008 (1 st clipping)	24 May - 19 June	16 June	---
	2008 (2 nd clipping)	21 June - 17 July	14 July	---
	2008 (3 rd clipping)	19 July - 14 August	12 August	12 August ¹
	2008 (4 th clipping)	20 October	20 October	20 October ¹
	2009	19 June	19 June	19 June

(b)	Plant materials	2007	2008
		‘Same-stage’ clipping regime	
	<i>P. spicata</i>		
	Anatone	5 May (30 April – 11 May)	31 May (22 May – 9 June)
	Goldar	12 May (30 April – 21 May)	31 May (22 May – 19 June)
	P-22	6 May (30 April – 11 May)	30 May (24 May – 9 June)
	P-26	6 May (30 April – 11 May)	31 May (22 May – 19 June)
	P-27	8 May (2 May – 11 May)	12 June (27 May – 19 June)
	<i>E. wawawaiensis</i>		
	Secar	13 May (2 May – 21 May)	5 Jun (28 May – 19 June)
	Discovery	14 May (5 May – 12 May)	6 June (22 May – 9 June)
	E-46	12 May (2 May – 16 May)	4 June (27 May – 12 June)
		‘Same-date’ clipping regime	
		21 May	16 June

** for specific date of emergence of each population see Table 1a.

¹ clipping on these dates not considered deleterious for given regime.

Table 2. Analysis of variance (F -values) for three morphological traits for five *P. spicata* (PSSP) and three *E. wawawaiensis* (ELWA) populations measured in the year of establishment (2006).

	df	Shoot biomass (g)	Tiller number	SLA (m ² kg ⁻¹)
Species	1	1.13	2.29	68.69***
Populations (species)	6	26.88***	35.94***	13.45***
<i>PSSP populations</i>	4	32.42***	53.50***	17.33***
<i>ELWA populations</i>	2	15.78***	0.81	5.62**

Table 3. Repeated measures ANOVA (F - values) for shoot biomass, defoliation tolerance, and specific leaf area (SLA) of five *P. spicata* (PSSP) and three *E. wawawaiensis* (ELWA) populations in response to spring defoliation under two clipping regimes.

	df	Shoot biomass (g) (2007-2009)	df	Defoliation tolerance (%) (2008 – 2009)	SLA (m ² kg ⁻¹) (May & October 2008)
Regime	1	4.84*	1	1.59	0.00
Period	2	99.46***	1	6.60*	887.83***
Regime*period	1	2.33 ⁺	1	0.02	0.00
Species	1	3.71 ⁺	1	26.43***	235.60***
Populations (species)	6	1.62	6	4.39**	10.63***
<i>PSSP populations</i>	4	1.88 ⁺	4	2.77*	14.73***
<i>ELWA populations</i>	2	1.09	2	0.71	2.02
Regime*species	1	1.33	1	1.53	0.10
Regime*population (species)	6	1.76	6	3.56**	0.78
<i>Regime*PSSP populations</i>	4	1.78	4	4.00**	0.11
<i>Regime*ELWA populations</i>	2	1.76	2	0.71	1.08
Period*species	1	19.50***	1	2.57	0.56
Period*population (species)	6	2.70**	6	2.98*	6.66***
Regime* period *species	1	0.82	1	1.20	1.42
Regime*period *population(species)	6	0.56	6	1.47	0.49
Defoliation	1	1770.16***	1	--	11.70**
Defoliation*period	2	249.78***	1	--	10.94**
Defoliation*species	1	36.33***	1	--	1.85
Defoliation*populations(species)	6	3.75**	6	--	1.27
<i>Defoliation*PSSP populations</i>	4	4.17**	4	--	1.81
<i>Defoliation*ELWA populations</i>	2	2.65 ⁺	2	--	0.01
Defoliation*period*species	1	7.42**	1	--	3.26 ⁺
Defoliation*period*population(species)	6	1.84**	6	--	1.04

⁺ P<0.10, *P < 0.05, **P < 0.01, ***P < 0.0001.

Table 4. Analysis of variance (*F*-values) for three morphological traits of five *P. spicata* (PSSP) and three *E. wawawaiensis* (ELWA) populations in response to spring defoliation (2008) under two clipping regimes.

	df	Tiller number (May 2008)	Leaf length (cm) (August 2008)	Plant basal area (cm ²) (August 2008)
Regime	1	0.74	1.50	5.42*
Species	1	0.80	36.98***	6.82**
Populations (species)	6	10.00***	2.14 ⁺	2.77*
<i>PSSP populations</i>	4	14.87***	2.84*	1.84
<i>ELWA populations</i>	2	0.30	0.83	4.61*
Regime*species	1	2.77 ⁺	0.15	1.09
Regime*population (species)	6	2.83*	0.89	0.52
<i>Regime*PSSP populations</i>	4	2.99*	0.54	0.59
<i>Regime*ELWA populations</i>	2	2.55 ⁺	1.57	0.58
Defoliation	1	112.29***	11.74**	248.16***
Defoliation*species	1	18.45***	0.39	13.17**
Defoliation*population (species)	6	2.04 ⁺	0.67	4.04**
<i>Defoliation*PSSP populations</i>	4	2.63*	0.72	4.09**
<i>Defoliation*ELWA populations</i>	2	0.76	0.47	1.27

⁺ P<0.10, *P < 0.05, **P < 0.01, ***P < 0.0001.

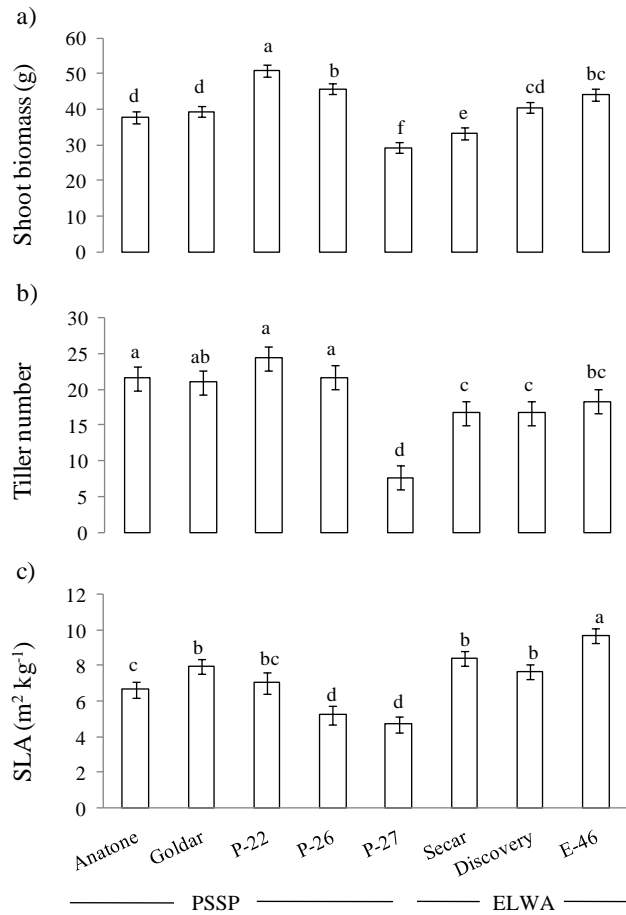


Figure 1. Means and standard errors for (a) annual shoot biomass, (b) tiller number, and (c) specific leaf area (SLA) for five *Pseudoroegneria spicata* (PSSP) and three *Elymus wawawaiensis* (ELWA) populations in the year of establishment (2006). Different letters represent significant ($p < 0.05$) differences among populations.

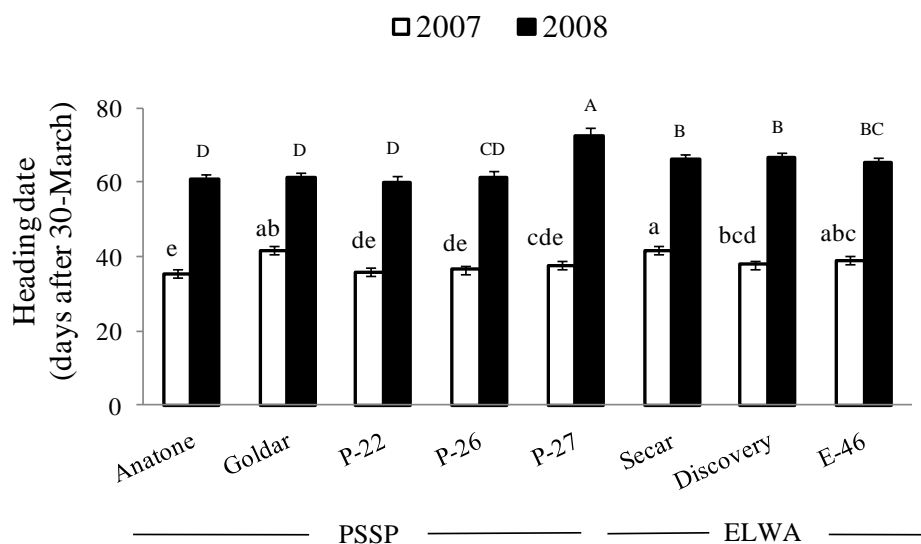


Figure 2. Means and standard errors for heading date for five *Pseudoroegneria spicata* (PSSP) and three *Elymus wawawaiensis* (ELWA) populations in the first (2007) and second (2008) years of spring defoliation. Different letters represent significant ($p < 0.05$) differences among populations within 2007 (lower case) and 2008 (upper case).

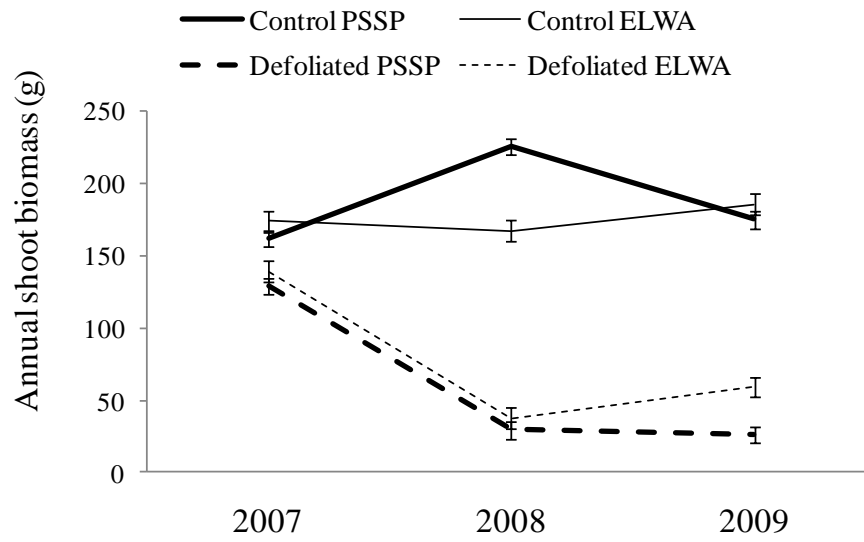


Figure 3. Mean annual shoot biomass for control and spring defoliation (applied in 2007 and 2008) treatments for five *Pseudoroegneria spicata* (PSSP) and three *Elymus wawawaiensis* (ELWA) populations.

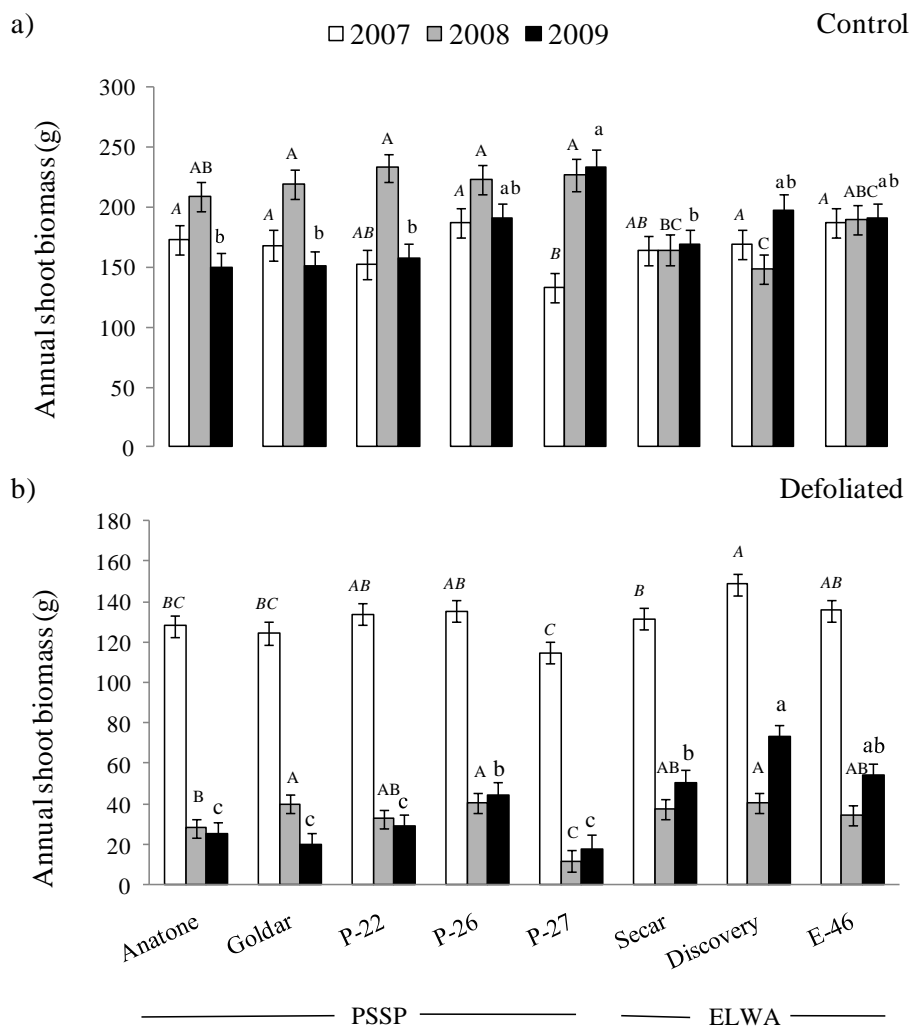


Figure 4. Means and standard errors for annual shoot biomass of five *Pseudoroegneria spicata* (PSSP) and three *Elymus wawawaiensis* (ELWA) populations from 2007 to 2009 for (a) control and (b) spring-defoliated treatments. Different letters represent significant ($p < 0.05$) differences among populations within 2007 (upper case italics), 2008 (upper case), and 2009 (lower case).

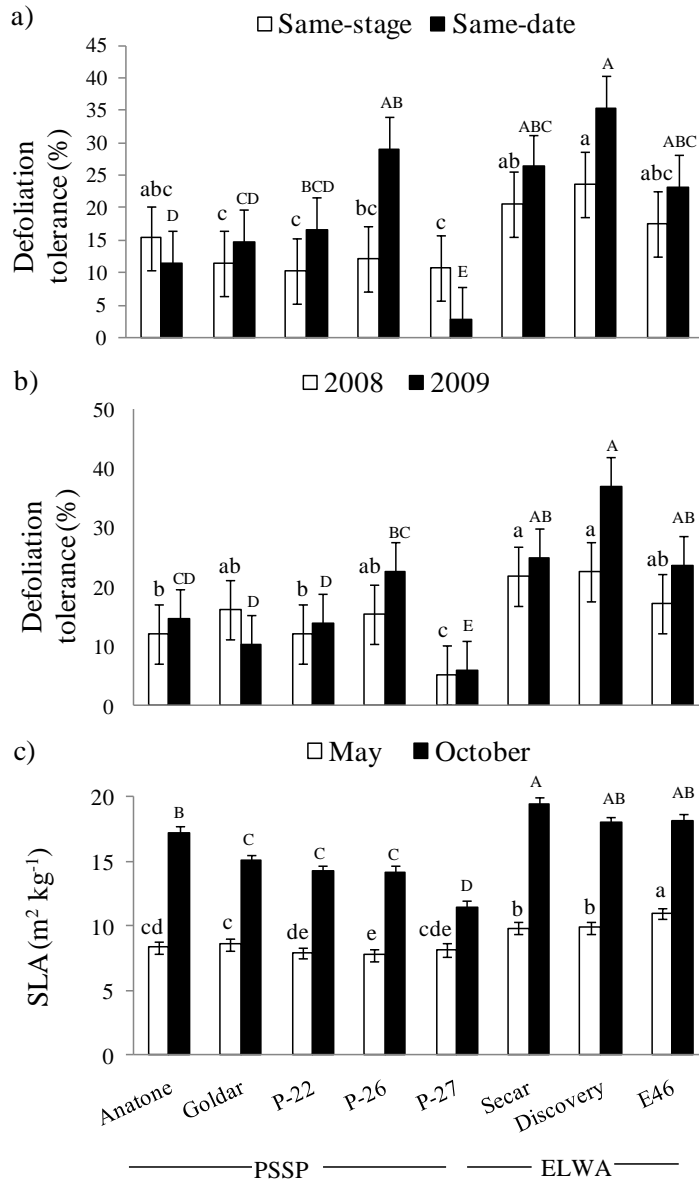


Figure 5. Means and standard errors for (a) defoliation tolerance (%) at two clipping regimes, (b) defoliation tolerance after one (2008) and two (2009) years of defoliation, and (c) specific leaf area (SLA) in May and October (2008) for five *Pseudoroegneria spicata* (PSSP) and three *Elymus wawawaiensis* (ELWA) populations. Different letters represent significant ($P < 0.05$) differences among populations under different clipping regimes at different time periods.

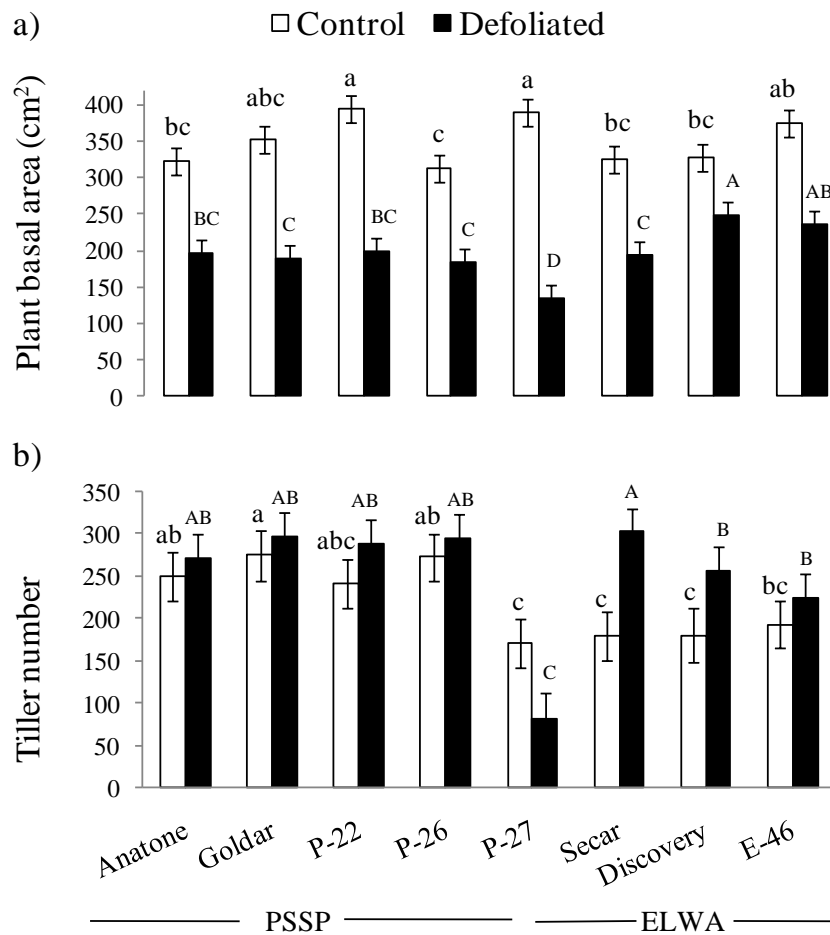


Figure 6. Means and standard errors for five *Pseudoroegneria spicata* (PSSP) and three *Elymus wawawaiensis* (ELWA) populations for (a) plant basal area (August 2008) and (b) tiller number (May 2008) for control and spring-defoliation treatments. Different letters represent significant ($p < 0.05$) differences among populations within control (lower case) and spring-defoliation (upper case) treatments.

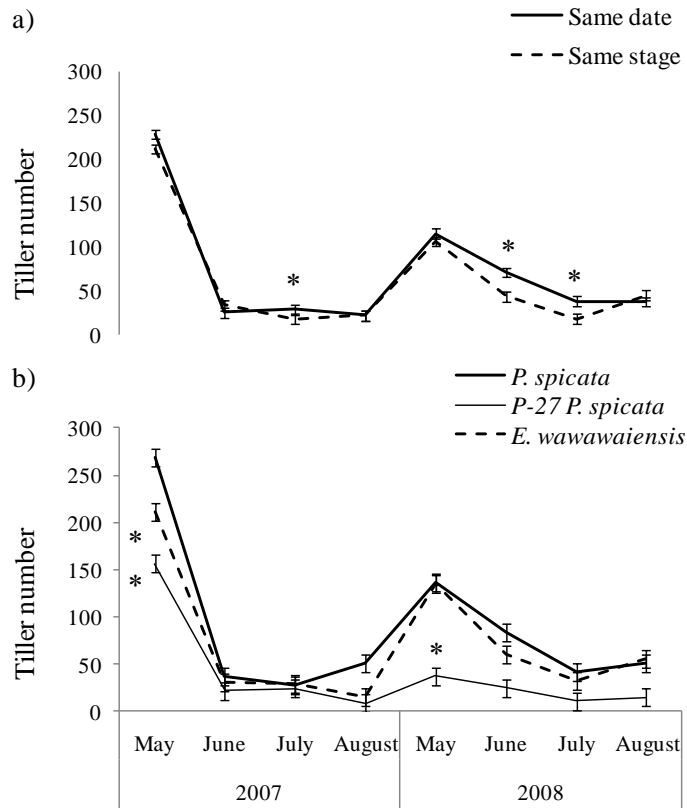


Figure 7. Means and standard errors under two clipping regimes for (a) tiller regrowth following each defoliation treatment in 2007 and 2008 and (b) tiller regrowth for four *P. spicata* populations, P-27 *P. spicata*, and three *E. wawawaiensis* populations in 2007 and 2008.

* indicates significant ($P < 0.05$) differences in tiller number.

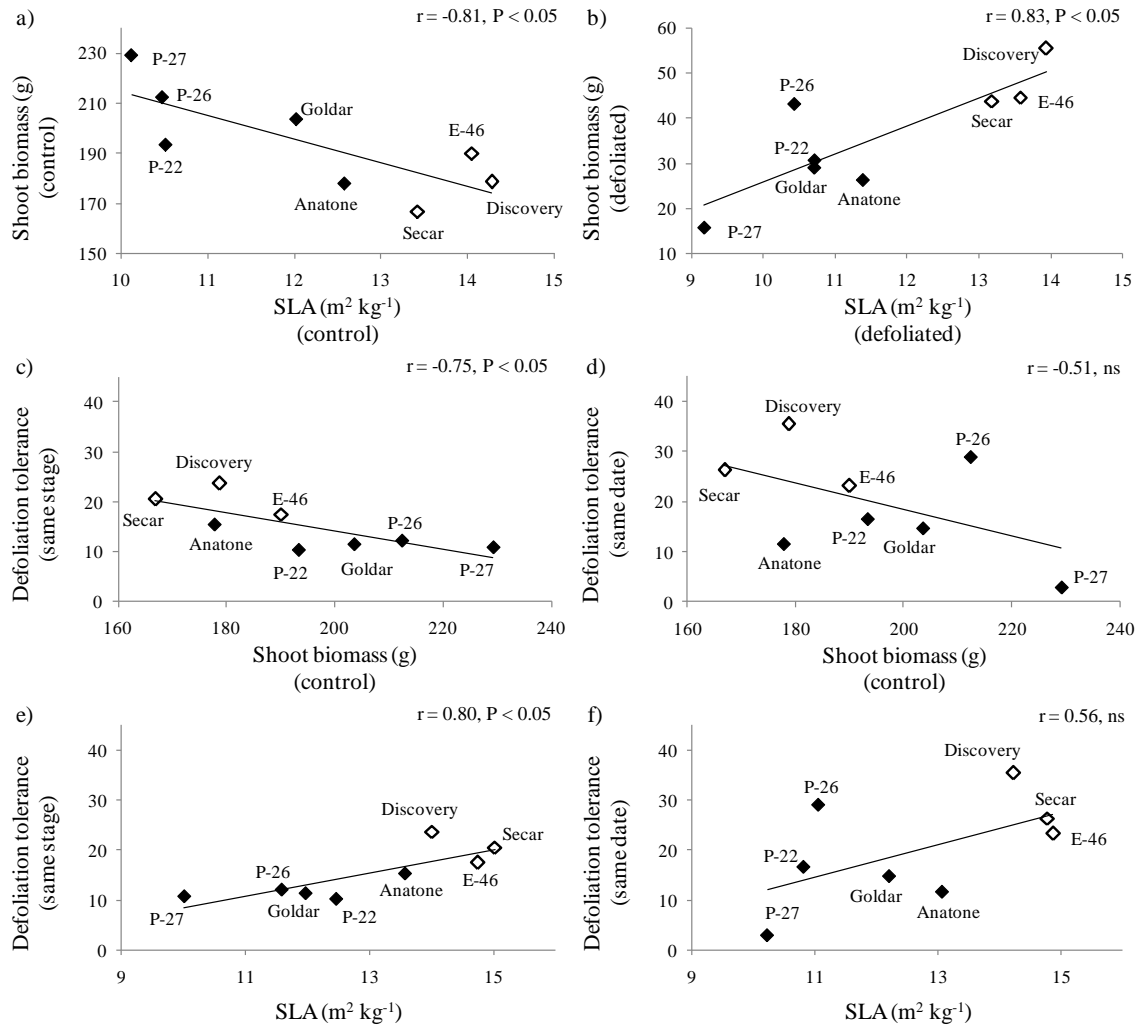


Figure 8. Correlations of (a) control shoot biomass with control SLA, (b) defoliated shoot biomass with defoliated SLA, (c) control shoot biomass with defoliation tolerance at 'same stage', (d) defoliated control shoot biomass with defoliation tolerance at 'same date', (e) SLA with defoliation tolerance at 'same stage', and (f) SLA with defoliation tolerance at 'same date' across five *Pseudoroegneria spicata* (◆) and three *Elymus wawawaiensis* (◇) populations.

CHAPTER 6

DIFFERENTIAL RESPONSE OF *PSEUDOROEGRNERIA SPICATA* POPULATIONS
TO DEFOLIATION AT TWO PLANT DENSITIES¹

Abstract

Pseudoroegneria spicata (Pursh.) A. Löve is an important native grass of the rangelands of the Intermountain West, USA and is widely used in the region for restoration applications. High grazing preference, together with high grazing sensitivity, is responsible for a decline in the abundance of this species, indicating the need for development of grazing-tolerant plant materials. While a population may be defoliation tolerant at low density, an effective grazing-tolerant population must also display this trait at higher densities where competition for limited soil resources is more acute. We compared two cultivars, two commercially available germplasms, and eight experimental populations of *P. spicata* for response to a single spring defoliation at high and low plant densities in a field experiment. We recorded shoot and root biomass and estimated defoliation tolerance as defoliated-to-control shoot-biomass ratio. On average, two consecutive years of spring defoliation reduced shoot biomass (g m^{-2}) per area by 19% compared to the undefoliated control, and this reduction was similar for the two densities. Two populations were able to compensate for shoot biomass after two years of spring defoliation, while the others were undercompensated, as is commonly seen for cool-season grasses in arid and semiarid regions. We found a negative correlation ($R^2 = 0.36$) between defoliation tolerance and control shoot-biomass production, suggesting a possible tradeoff between growth and defoliation tolerance among populations. Of the commercially available *P. spicata* populations in our study, the more recently released

¹ Coauthored by: Jayanti Ray Mukherjee, TA Jones, and TA Monaco

germplasms, P-7 and Anatone, exhibited higher defoliation tolerance than the previously released cultivars, Whitmar and Goldar.

Introduction

Past overgrazing by livestock is one of the pivotal reasons for degradation of native-plant communities on the rangelands of the Intermountain West (Whisenant 1999). The native bunchgrasses in this region are grazing sensitive, probably due to low evolutionary grazing pressures from native herbivores prior to European settlement (Mack and Thompson 1982; Loreti et al. 2001). This lack of innate grazing tolerance heightens the importance of evaluating plant materials intended for this region for defoliation tolerance. *Pseudoroegneria spicata* (Pursh.) A. Löve is a C₃, perennial Triticeae bunchgrass that occurs widely in this region and is an important source of forage (Monsen et al. 2004). In general, this species is believed to be grazing sensitive compared to other exotic (Caldwell et al. 1981) and native (Jones and Nielson 1997) perennial grasses. Past overgrazing of the highly preferred and grazing sensitive grass has caused a decline in its abundance (Jones and Nielson 1997; Adler et al. 2005).

Both theoretical and empirical studies have shown that plants sometimes have the ability to compensate for tissue lost to defoliation, as remaining tissues can perform more efficiently (McNaughton 1983; Del-Val and Crawley 2005). But in agricultural systems and in semi-arid to arid rangelands, plants often fail to replace lost tissue loss and remain undercompensated (Trlica and Rittenhouse 1993). Moreover, the negative effect of defoliation may be amplified when density is high (Lee and Bazzaz 1980). Under such circumstances, intraspecific competition, which causes resource limitation, can greatly reduce yield per plant (Banyikwa 1988) and individual leaf biomass (Archer and Detling

1984). Thus, it is important to study the interactive effects of density and defoliation (Banyikwa 1988) and to understand how these effects vary with population. If density interacts with defoliation tolerance in some populations, this complicates the selection of populations for defoliation tolerance. A desirable defoliation-tolerant plant material would be one that maintains relatively high shoot biomass despite defoliation at high density.

In natural rangelands, where plants occur at higher densities than under typical spaced-plant experimental conditions, even mild defoliation can severely damage *P. spicata* in the presence of competing neighbors. This grass may require six to eight years to completely recover once heavily grazed (Mueggler 1975). In spite of its grazing sensitivity, *P. spicata* continues to be a preferred species for restoration applications in the Intermountain West (Monsen et al. 2004). Two released cultivars of *P. spicata*, Goldar and Whitmar, and two pre-variety germplasms, Anatone and P-7, are commercially available for contemporary restoration practice. In restoration applications, seeding rates of native species range from 100 to 300 seeds m⁻², although establishment success under natural rangeland conditions is on the order of about 10 seedlings m⁻² (Whisenant 1999). Therefore, greater tolerance to defoliation at high densities where resource is limited will result in greater persistence under rangeland conditions (Sheley et al. 2008).

Defoliated-to-control shoot-biomass ratio has been used to estimate defoliation tolerance (Jones and Nielson 1997). In this study, we wished to compare four commercially available (named above) and eight experimental populations of *P. spicata* for response to spring defoliation at high and low plant densities. *Pseudoroegneria*

spicata's sensitivity to grazing has been attributed to its slow recovery with respect to shoot biomass production (Mueggler 1975; Caldwell et al. 1981) and unabated root biomass production despite high defoliation intensities (Caldwell et al. 1981). To this end, we compared above- and below-ground biomass production for these populations to evaluate how a population's biomass production and defoliation tolerance vary across densities. Specifically, we wished to determine how shoot and root biomass are impacted by defoliation and density treatments and to describe the differential impact of these treatments on the 12 *P. spicata* populations.

Being a cool-season perennial grass, we expected that spring defoliation would be deleterious to *P. spicata* populations and that all populations would undercompensate for regrowth after two consecutive years of defoliation treatment. Because plant-material developers have evaluated *P. spicata* without applying defoliation, we expected that the more recently released germplasms of *P. spicata*, Anatone (released in 2003) and P-7 (2001), would be more productive for undefoliated controls than the earlier released cultivars, Whitmar (1946) and Goldar (1989). Plant ecophysiological studies have suggested that there is an evolutionary trade-off between genotypes' ability to grow and tolerate resource shortages (Chapin 1980; Grime 2001). Therefore, when biomass is reduced following defoliation and resources remain unchanged, we expected that less-productive populations would be better able to compensate and display greater defoliation tolerance at high, as well as low, densities. Grazing-sensitive grasses are known to invest more in root biomass than grazing-tolerant grasses (Caldwell et al. 1981). Hence, we anticipated that defoliation-tolerant populations would respond to defoliation by reducing root biomass more than defoliation-susceptible populations.

Materials and methods

We used four released commercial (Goldar, Whitmar, Anatone, and P-7) and eight experimental (P-1, P-22, A-238, P-24, P-26, P-3, P-27t, and P-9t) *P. spicata* populations for this study. All populations are diploid ($2n = 14$) except P-27t and P-9t, which are tetraploid ($2n = 28$), as indicated by terminal 't'. P-1 is a population of unknown origin, and P-22 was developed from P-1 by four cycles of selection, one of which was for recovery after clipping. A-238 is a population collected near Lind, WA, and P-24 was developed from A-238 by three cycles of selection with no emphasis on clipping tolerance. P-26 was developed by three cycles of selection from P-7, a genetically diverse polycross developed from 25 intermating populations, again with no emphasis on clipping tolerance. P-3 is a polycross from several populations collected along the Grande Ronde River in northeastern Oregon and southeastern Washington, and P-27t is a colchicine-induced tetraploid derived from P-3. P-9t is a colchicine-induced tetraploid derived from the diploid population, P-5, which in turn was developed by two cycles of selection from P-1.

Seeds of these populations were germinated in plastic boxes under laboratory conditions. A week after adding water, seedlings were transplanted into a 3:1 sand:peat moss mixture in opaque, round plastic tubes (20-cm long and 5-cm wide). Seedlings were reared in a greenhouse on the Utah State University campus in Logan, Utah. After about three months, seedlings of the 12 populations were transplanted on 18 and 19 May 2005 to Millville, Utah at two densities. Low-density plots had five plants per plot (6400 cm^2), with four corner plants spaced 60-cm apart and one plant in the center, and high-density plots had 16 plants per plot (6400 cm^2) in a 4-by-4 square arrangement, with each plant

20 cm from its nearest neighbor. Our low-density plots (8 plants m⁻²) represented a density at which rangeland plants display optimal establishment (Whisenant 1999), while our high-density plots (25 plants m⁻²) represented a density intermediate between optimal establishment and typical seeding rates. A 40-cm aisle surrounded each plot on all sides, and a single-row border was maintained around the perimeter of the plot area 40 cm from the nearest plot edge. Soil at the Millville site was a Ricks gravelly loam (coarse-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Calcic Haploxerolls). Shortly after transplanting the seedlings to Milville, they were irrigated only once in 2005, the establishment year.

On 8 May 2006, at the pre-anthesis stage, i.e., when the spike was fully exerted but prior to pollen shed, we applied the first spring defoliation to the whole plots (low and high density) designated for the defoliation treatment. Biomass was oven-dried at 60°C for 72 hrs, and dry weights were recorded. Stomatal conductance was measured on 5 and 6 June on the center plant of low-density plots and on any of the four center plants of high-density plots using a leaf porometer (Model SC-1, Decagon Devices, Pullman, WA). We harvested regrowth of defoliated plots and reproductive biomass (with matured seeds) on control plots on 15 July. Fall-regrowth was harvested on both defoliation treatments on 11 November.

On 31 May 2007, we defoliated the same set of whole plots as in May 2006, again when plants were at the pre-anthesis stage. We measured stomatal conductance on 25 and 26 June 2007 using same procedure as in 2006. On 31 July we harvested biomass of both defoliated and control whole plots. Biomass was oven dried for dry weight. At the end of the experiment in the next year (2008), we harvested biomass from defoliation and

control whole plots on 31 May. We harvested biomass from all plots for the last time on 2 September 2008. From 9 to 13 September, we subsampled three replications by removing four soil cores (10-cm diameter x 17-cm height, 1450 cm³), each approximately 15 cm from the center of the plot in each direction, using a golf-cup cutter (H 10 Hole cutter, KSAB Gold Equipment, Västerås, Sweden). We cleaned root samples, refrigerated, and cleaned again before scanning with WinRHIZO Pro Version 2005b (Reagent Instrument Inc., Québec City, Canada). Root length density (root length per volume of soil), average root diameter, and specific root length (length per biomass) were calculated on these samples, and roots were oven dried at 60° C for 48 hrs and weighed.

To summarize, for all defoliations, shoot biomass was removed at a 10-cm height from all individuals within the plot. The defoliation treatment received a spring defoliation at the time when plants were at the early-reproductive phase, when the effect of defoliation is most deleterious (Ganskopp 1988; Sheley et al. 1997; Jones and Nielson 1997). This was followed with a defoliation in mid-summer (2006 and 2007) and late fall (2006 only). In contrast, the undefoliated controls received only the mid-summer and fall defoliations, when deleterious effects are minimal. No defoliation was applied in fall 2007, as regrowth was minimal due to drought conditions (US Drought Monitor 2010).

A total of 288 plots across 12 populations of *P. spicata*, two plant densities, and two defoliation treatments were arranged in a split-split plot design with six replicated blocks. Defoliation treatment was the whole-plot factor, density was the split-plot factor, and populations were the split-split-plot factor. All effects were considered fixed except replication. Shoot biomass, defoliation tolerance, and root biomass data were analyzed using PROC MIXED and PROC REG in SAS 9.1.3 (SAS Institute 2003). Normality

assumptions were not met for defoliated-to-control shoot-biomass ratio (defoliation tolerance), so we log-transformed these data. A repeated-measure ANOVA was performed on shoot biomass. When three-way and two-way interactions were not significant, they were removed from the model. We separated least-squares means with LSD at $P < 0.05$. Number of surviving plants was not independent of the density treatment, and hence it was not used as a covariate. Instead, we also analyzed shoot biomass per plant for each density with PROC MIXED.

Results

For shoot biomass totaled across the three years (2006-2008), the control treatment was 19% greater than the defoliated treatment ($P < 0.01$), and the high-density treatment was 8% greater than the low-density treatment ($P < 0.0001$) (Table 1). Populations varied significantly ($P < 0.0001$) for three-year shoot biomass and interacted with defoliation treatment ($P < 0.0001$), but not with density (Table 1). Shoot biomass for the control treatment was greatest for P-7, P-26, P-1, and P-9t, while P-24 and A-238 exhibited the least control shoot biomass (Fig. 1). Under defoliation, P-7, P-26, and P-3 had greater biomass than the other *P. spicata* populations. For annual shoot biomass, density, defoliation, and population interacted with year (Table 1), but none of the three-way interactions were significant. While defoliation increased shoot-biomass production 7% in 2006, the first year of defoliation, defoliation reduced shoot biomass 35% in 2007 and 14% in 2008. Compared to the low-density treatment, the high-density treatment produced 40% greater shoot biomass per plot in 2006 and 4% greater shoot biomass per plot in 2008, while in 2007 the two densities were similar.

Across all populations, average biomass per plant at low density was 33.8 g plant⁻¹ in 2006, 62.2 g plant⁻¹ in 2007, and 41.2 g plant⁻¹ in 2008 (Table 2, Fig. 2). Fluctuations between years were much lower for high density, where average biomass per plant was 15.2 g plant⁻¹ in 2006, 18.7 g plant⁻¹ in 2007, and 13.7 g plant⁻¹ in 2008. Average biomass per plant was 55.0%, 66.9%, and 66.6% greater at low density than high density in 2006, 2007, and 2008, respectively ($P < 0.0001$).

Defoliation tolerance, estimated as defoliation-to-control shoot-biomass ratio, did not vary with density, but populations significantly ($P < 0.01$) differed for this trait (Table 1). The population P-3 displayed the highest defoliation tolerance (Fig. 3), significantly higher than Whitmar, Goldar, P-26, P-1, and P-9t. Populations were similarly defoliation tolerant at the two densities. On average, defoliation tolerance differed for the three years ($P < 0.0001$), and populations also varied with years ($P < 0.01$) for this variable. However, just four of the populations, Anatone, P-26, P-24, and P-27t, were responsible for this interaction. These populations exhibited a significant reduction in defoliation tolerance from 2006 to 2007, while they remained unchanged in the following year (2008). The other populations displayed similar defoliation tolerance across years. Regression of defoliated biomass on control biomass was only marginally positive ($R^2 = 0.26$) ($P < 0.10$) (Fig 4a), and regression of defoliation tolerance on control shoot biomass was negative ($R^2 = 0.36$) ($P < 0.05$) (Fig. 4b).

Root biomass on a plot basis was significantly ($P < 0.01$) affected by density (Table 3), and low density had 52% lower root biomass than high density. Defoliation and density interacted ($P < 0.0001$) for root biomass, and at high density, defoliation reduced root biomass by 30.0%, while at low density root biomass was not affected by

defoliation. Populations varied ($P < 0.005$) for root biomass and interacted ($P < 0.05$) with density but not with defoliation (Table 3). At high density, P-1, P-22, P-26, Whitmar, P-3, and Anatone produced the greatest root biomass, while P-9t and A-238 produced the least. At low density, P-7, P-26, Whitmar, and Anatone produced the greatest biomass, while A-238 produced the least (Fig. 5). At high density, we found a positive correlation ($R^2 = 0.60$) (Fig. 6a) across populations between reductions in shoot and root biomass by defoliation. However, we found no such relationship ($P > 0.05$) at low density (Fig 6b).

At high density, defoliation reduced root diameter 31.2% ($P < 0.10$), while at low density defoliation increased root diameter 19.3%. At high density, defoliation reduced root length density 32.7%, while at low density, defoliation reduced root length density by only 10.9% ($P < 0.10$) (Table 3). On the other hand, specific root length was similar among defoliation treatments, density treatments, and populations (Table 3). Stomatal conductance was 100.8% greater for the defoliation treatment regardless of density ($P < 0.01$) (Table 1). In 2006, stomatal conductance was 35.2% greater at low density than high density ($P < 0.05$), while in 2007 stomatal conductance was similar for high and low densities.

Discussion

Previous studies have demonstrated that defoliation is detrimental to *P. spicata* in May, the peak growing period for these semi-arid rangelands (Sims and Singh 1978), for both immature (Brewer et al. 2007) and mature plants of *P. spicata* (Richards and Caldwell 1985; Ganskopp 1988). Consistent with previous studies (Cook and Stoddard 1963; Ganskopp 1988; Sheley et al. 1997), we found that spring defoliation significantly

impacted plant performance and elicited differential response among *P. spicata* populations. Although defoliation increased shoot biomass in the first year when plants were still establishing, two consecutive years of spring defoliation had a more negative effect on biomass production.

Shoot biomass on a g plant^{-1} basis was greater for low density than high density in all three years. Also, at low density, root variables like root biomass, root diameter, and root length density were not affected by defoliation, while at high density these traits were negatively impacted by defoliation. The greater stomatal conductance in 2006 for low-density plots also supports this argument, although this was not true for 2007. The effect of defoliation on biomass per plant is important for understanding how defoliation impacts competitive interactions and which plant traits and physiological mechanisms effect such changes. On the other hand, the objective for restoration applications and management is to understand how biomass production of *P. spicata* populations per area is affected by defoliation. Defoliation did not affect shoot-biomass production on a per-area basis in the first year that the treatment was applied (2006), but the effect of defoliation was present in 2007 and even more pronounced in 2008. Density, on the other hand, had a significant effect on per-area shoot biomass in 2006, but densities did not differ for per-area shoot biomass in the latter two years. This means that the biomass of five plants (low density) was equivalent to that of 16 plants (high density) in 2007 and 2008. The effect of density on shoot biomass on a per-area basis declined over time because of negative density-dependence, which reduced biomass per plant more at high densities than at low densities (Yoda et al. 1963; Westoby 1984; Silvertown 1987). Here, we report a significant year x density interaction for shoot biomass, indicating that

negative density dependence was present. However, we found no year x population x density interaction for shoot biomass, indicating that the negative effect of density was similar across populations.

P-3, P-24, and A-238 displayed the highest defoliation tolerance at both densities. P-3 and A-238 were the only populations that exhibited full compensation for shoot biomass after two years of mild spring defoliation, while all other populations remained undercompensated. This is in accordance with Belsky's (1986) assertion of undercompensation for species of arid and semi-arid grasslands, yet it is also consistent with our prediction that less-productive populations would be able show greater compensation at high density, as well as at low density. Among the four commercialized populations, P-7 was most productive with and without defoliation, while Anatone was more similar to Whitmar and Goldar. However, Anatone and P-7 were similar for defoliation tolerance and better than Whitmar and Goldar, indicating that P-7 and Anatone may be more desirable for grazing lands.

The correlation between control and defoliation shoot biomass among populations was positive and largely due to the exceptional performance of P-7 and P-26, a population derived from P-7, which were productive under both defoliation treatments. In addition, a significant negative association between control-shoot biomass and defoliation tolerance indicates that populations more productive for the control displayed less defoliation tolerance. This suggests the possibility of a tradeoff between growth and tolerance within *P. spicata* (Grime 2001). However, P-7 seems to be an exception to the observed trend, because despite its high productivity it experienced only a slight reduction in shoot biomass with defoliation, implicating that this population is able to

overcome this tradeoff at lower levels of stress. *Pseudoroegneria spicata* lacks an evolutionary history of exposure to heavy grazing, but the unusually high defoliation tolerance of A-238 and P-24, the two least-productive populations, may have been conditioned by arid environmental conditions (250 mm average annual precipitation) of the A-238 collection site, which is thought to promote grazing resistance (Coughenour 1985; Milchunas et al. 1988; Adler et al. 2004).

Differential population response for shoot biomass was primarily due to defoliation rather than density, while the reverse was true for root biomass. This may be intuitive, as defoliation primarily alleviates above-ground competition, and low density primarily alleviates below-ground competition. Compared to *Agropyron desertorum* (Fisch. ex Link) Schult., a grazing-tolerant, exotic perennial bunchgrass, *P. spicata* is known for its unabated root growth (Caldwell et al. 1981), even after severe defoliation (Richards 1984). This suggests the hypothesis that curtailing root growth may be an effective strategy for defoliation tolerance. If this hypothesis is correct, a negative slope should result when reductions in root biomass of individual populations are regressed on their reductions in shoot biomass following defoliation. However, as mentioned earlier, our study exhibited a positive slope for this regression at high density, while no relationship was seen for low density. This suggests that, in highly competitive environments, populations with greater reductions in root biomass also realized greater reductions in shoot biomass and vice-versa.

This finding is inconsistent with previous studies (Caldwell et al. 1981; McNaughton et al. 1998), and the reason might be that at high density the effect of defoliation on roots may have been overshadowed by greater density effects, impacting

shoot and root production similarly. Greater root-biomass production at high density, seen here for P-1, Anatone, Whitmar, P-22, P-26, and P-3, could be an indicator of greater competitive ability (Grime 2001), and the greater shoot and root biomass of P-7 may allow this population to be both defoliation tolerant and competitive.

To summarize, we found intraspecific variation among populations for defoliation tolerance, but only two populations (P-3 and A-238) were able to compensate for shoot biomass removal. *Pseudoroegneria spicata* is known as a grazing-sensitive species, and, according to our results, among the commercially released plant materials, Goldar and Whitmar are particularly defoliation sensitive. Although Anatone and P-7 revealed higher and similar tolerance to defoliation, Anatone was less productive than P-7 for the control. The two least-productive populations, A-238 and P-24, derived from A-238, deserve special mention for their high defoliation tolerance.

In our study, defoliation tolerance did not differ between the two densities and neither did density interact with *P. spicata* populations or years. While defoliation tolerance is generally considered to be greater in the absence of plant competition (Lee and Bazzaz 1980; Archer and Detling 1984; Banyikwa 1988), in our case any possible differences in resource availability were inadequate to generate differences in defoliation tolerance. While density had no discernible effect on production of biomass per area or defoliation tolerance, it had a large impact on root biomass, though this could be partly be explained by the differences in planting pattern of the two densities relative to the holes dug to harvest root samples. Nevertheless, density also interacted with defoliation and *P. spicata* populations for root biomass. These results suggest that density had a greater impact on resource availability to roots than shoots.

Defoliation tolerance was negatively associated with productivity of the undefoliated control, indicating a trade-off between defoliation tolerance and growth among these *P. spicata* populations, which was consistent at the two densities. We agree with Del-Val and Crawley (2005) that moderate defoliation under experimental conditions cannot be extrapolated to responses of recurrent herbivory in the field. But our comparative study clearly shows that the populations of *P. spicata* currently used for most restoration applications are grazing sensitive even under the moderate levels of defoliation applied in this experiment. Because we found intraspecific variation for defoliation tolerance, developing future plant materials with higher levels of defoliation tolerance appears to have potential in this species. From this study, we conclude that under natural rangeland conditions, moderate to light defoliation for two consecutive years could be damaging to *P. spicata*. Hence, as recent studies suggest (Brewer et al. 2007), multi-year rotational grazing would be more desirable, allocating more time for recovery.

References

- ADLER, P. B., D. G. MILCHUNAS, O. E. SALA, I. C. BURKE, AND W. K. LAUENROTH. 2005. Plant traits and ecosystem grazing effects: comparison of U.S. sagebrush steppe and Patagonian steppe. *Ecological Applications* 15: 774–792.
- ADLER, P. B., D. G. MILCHUNAS, W. K. LAUENROTH, O. E. SALA, AND I. C. BURKE. 2004. Functional traits of graminoids in semi-arid steppes: a test of grazing histories. *Journal of Applied Ecology* 41: 653–663.

- ARCHER, S., AND J. K. DELTING. 1984. The effect of defoliation and competition on the growth of tillers of two North American mixed-grass prairie graminoids. *Oikos* 43: 351-357.
- BELSKY, A.J. 1986. Does herbivory benefit plants? A review of the evidence. *The American Naturalist* 127: 870-892.
- BANYIKWA, F. F. 1988. The growth response of two East African perennial grasses to defoliation, nitrogen fertilizer, and competition. *Oikos* 51: 25-30. BELSKY, A. J. 1986. Does herbivory benefit plants? A review of the evidence. *The American Naturalist* 127: 870-892.
- BREWER, T. K., J. C. MOSLEY, D. E. LUCAS, AND L. R. SCHMIDT. 2007. Bluebunch wheatgrass response to spring defoliation on foothill rangeland. *Rangeland Ecology and Management* 60: 498-507.
- CALDWELL, M. M., J. H. RICHARDS, D. A. JOHNSON, R. S. NOWAK, AND R. S. DZUREC. 1981. Coping with herbivory: photosynthetic capacity and resource allocation in two semi-arid *Agropyron* bunchgrasses. *Oecologia* 50: 14-24.
- CHAPIN, F. S. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* 11: 233-260.
- COOK, C. W., AND L. A. STODDART. 1963. The effect of intensity and season of use on the vigor of desert range plants. *Journal of Range Management* 16: 315-317.
- COUGHENOUR, M. B., S. J. MCNAUGHTON, AND L. L. WALLACE. 1985. Responses of an African tall-grass (*Hyparrhenia filipendula* stapf.) to defoliation and limitations of water and nitrogen. *Oecologia* 44: 530-542.

- DEL-VAL, E., AND M. J. CRAWLEY. 2005. What limits herb biomass in grasslands: competition or herbivory? *Oecologia* 142: 202–211.
- GANSKOPP, D. 1988. Defoliation of Thurber needlegrass: herbage and root responses. *Journal of Range Management* 41: 472–476.
- GRIME, J. P. 2001. Plant strategies, vegetation processes, and ecosystem properties. New York, USA: John Wiley & Sons. 460 p.
- JONES, T. A., AND D. C. NIELSON. 1997. Defoliation tolerance of bluebunch and Snake River wheatgrasses. *Agronomy Journal* 89: 270 – 275.
- LEE, T. D., AND F. A. BAZZAZ. 1980. Effects of defoliation and competition on growth and reproduction in the annual plant *Abutilon theophrasti*. *Journal of Ecology* 68: 813–821.
- LORETI, J., M. OSTERHELD, AND S. OSVALDO. 2001. Lack of intraspecific variation in resistance to defoliation in a grass that evolved under light grazing pressure. *Plant Ecology* 157: 195–202.
- MACK, R. N., AND J. N. THOMPSON. 1982. Evolution in steppe with few large, hooved mammals. *The American Naturalist* 119: 757–773.
- MCNAUGHTON S. J., F. F. BANYIKWA, AND M. M. MCNAUGHTON. 1998. Root biomass and productivity in a grazing ecosystem. *Ecology* 79: 587–592.
- MCNAUGHTON, S. J. 1983. Compensatory plant growth as a response to herbivory. *Oikos* 40: 329–336.
- MILCHUNAS, D.G., O. E. SALA, AND W. K. LAUENROTH. 1988. A generalized model of the effects of grazing by large herbivores on grassland community structure. *The American Naturalist* 132: 87–106.

- MONSEN, S. B., R. STEVENS AND N. L. SHAW. 2004. Restoring western ranges and wildlands. U. S. Forest Service Gen. Tech. Rep. RMRS-GTR 136 (Vol. 2) Fort Collins, Colorado, USA
- MUEGGLER, W. F. 1975. Rate and pattern of vigor recovery in Idaho fescue and bluebunch wheatgrass. *Journal of Range Management* 28: 198–204.
- RICHARDS, J. H. 1984. Root growth response to defoliation in two *Agropyron* bunchgrasses: field observations with an improved root periscope. *Oecologia* 64: 21–25.
- RICHARDS, J. H., AND M. M. CALDWELL. 1985. Soluble carbohydrates, concurrent photosynthesis and efficiency in regrowth following defoliation: a field study with *Agropyron* species. *Oecologia* 64: 21–25.
- SAS [COMPUTER PROGRAM]. 2003. SAS Version 9.1.2. Cary, NC, USA: SAS Institute, Inc.
- SHELEY, R. L., B. E. OLSON, AND L. L. LARSON. 1997. Effect of weed seed rate and grass defoliation level on diffuse knapweed. *Journal of Range Management* 50: 39–43.
- SHELEY, R. L., B. S. BINGHAM, AND T. J. SVEJCAR. 2008. Crested wheatgrass defoliation intensity and season on medusahead invasion. *Rangeland Ecology and Management* 61: 211–217.
- SILVERTOWN, J. 1987. Introduction to plant population ecology. New York, NY, USA: John Wiley and Sons. 229 p.
- SIMS P. L. AND J. S. SINGH. 1978. The structure and function of ten western North American grasslands. III. Net primary production, turnover, and efficiencies of energy capture and water use. *Journal of Ecology* 66: 573–597.

TRLICA M.J. AND L.R. RITTENHOUSE. 1993. Grazing and plant performance. *Ecological Applications* 3: 21-23.

US DROUGHT MONITOR. 2010. Available at:

<http://www.ncdc.noaa.gov/img/climate/research/2007/sep/usdm-070925.gif>.

Accessed 10 Jan. 2010.

WESTOBY, M. 1984. The self-thinning rule. *Advances in Ecological Research* 51: 25-30.

WHISENANT, S. G. 1999. Repairing damaged wildlands: a process-oriented, landscape-scale approach. Cambridge, UK: Cambridge University Press. 312p.

YODA, K. T. KIRA, H. OGAWA, AND K. HOZUMI. 1963. Intraspecific competition among higher plants. XI. Self-thinning in over-crowded pure stands under cultivated and natural conditions. *Journal of Biology of Osaka City University* 51: 25-30.

Table 1. *F*-values of repeated-measures analysis of variance for shoot biomass production, defoliation tolerance, and stomatal conductance for 12 *P. spicata* populations in response to two defoliation treatments and two plant densities.

Effects	Shoot biomass (g m ⁻²) (2006 – 2008)	Defoliation tolerance (%) (2007 – 2008)	Stomatal conductance (mmol m ⁻² s ⁻¹) (2006 – 2007)
Defoliation	61.34**	---	31.20**
Density	20.42***	0.16	22.71**
Defoliation*density	0.02	---	0.23
Populations	15.31***	2.07*	1.11
Population*defoliation	5.20***	---	0.81
Population*density	1.15	0.70	0.54
Population*defoliation*density	0.75	---	0.74
Year	218.93***	68.69***	106.69***
Year*defoliation	78.06***	---	0.38
Year*density	39.56***	0.00	4.89*
Year*defoliation*density	0.06	---	2.70
Year*populations	2.55**	1.13	0.44
Year*defoliation*population	1.02	---	0.98
Year*density*population	0.56	0.53	0.64
Year* defoliation*density *population	0.34	---	0.75

P < 0.05, **P < 0.01, ***P < 0.0001

Table 2. *F* – values and *P* – values for ANOVA of shoot biomass per plant at low and high densities for 2006, 2007, and 2008.

Effects	<i>df</i>	<i>F</i> -values
Defoliation	1	57.66**
Density	1	1829.27***
Defoliation*density	1	0.39
Population	11	10.08***
Defoliation*population	11	3.11**
Density*population	11	1.82*
Defoliation*density*population	11	0.54
Year	2	157.06***
Year*defoliation	2	50.11***
Year*density	2	34.68***
Year*population	22	2.58***
Year*defoliation*density	2	0.34
Year*defoliation*year	22	0.64
Year*density*population	22	0.59
Year*defoliation*density*population	22	0.36

$P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$

Table 3. *F*- values for analysis of variance of four morphological root traits of *P. spicata* populations (P) in response to defoliation (DF) and density (DN).

Traits	df	DF	DN	DF*DN	P	DF*P	DN*P	DN*DF*P
Root biomass (g)	3	6.41	49.22**	27.40***	3.16**	1.19	2.17*	0.74
Root diameter (mm)	3	1.13	35.56**	7.82 ⁺	0.35	0.67	0.67	1.36
Root length density (m cm ⁻³)	3	3.36	32.38**	8.24 ⁺	0.35	0.86	0.52	1.42
Specific root length (m g ⁻¹)	3	0.20	3.51	0.19	1.37	0.83	0.66	0.65

⁺ P<0.10 * P < 0.05, **P < 0.01, ***P < 0.0001.

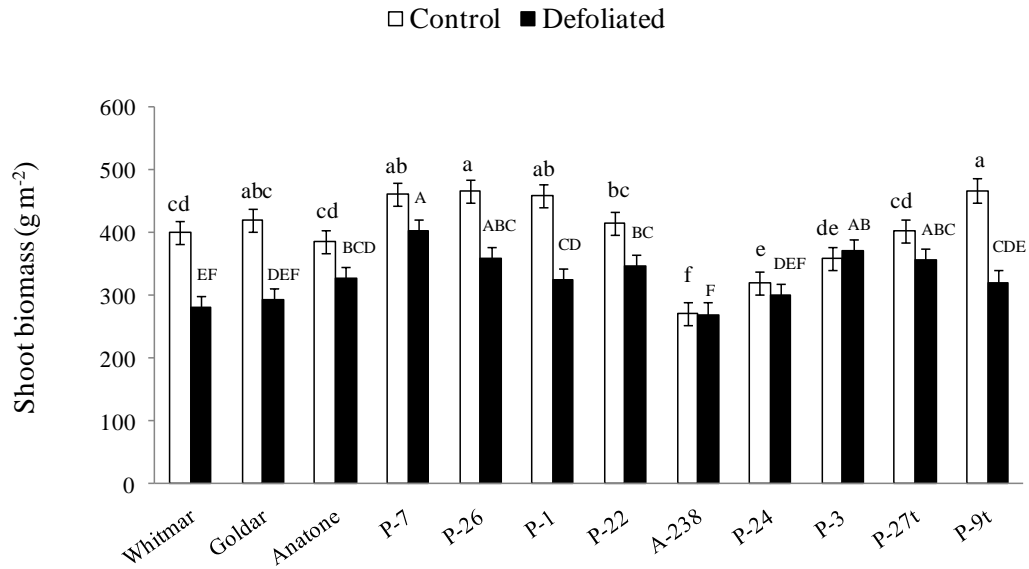


Figure 1. Means and standard errors of 12 *P. spicata* populations for average annual shoot biomass under control and defoliated treatments. Different letters represent significant ($p < 0.05$) differences among populations within control (lower case) and defoliated (upper case) treatments.

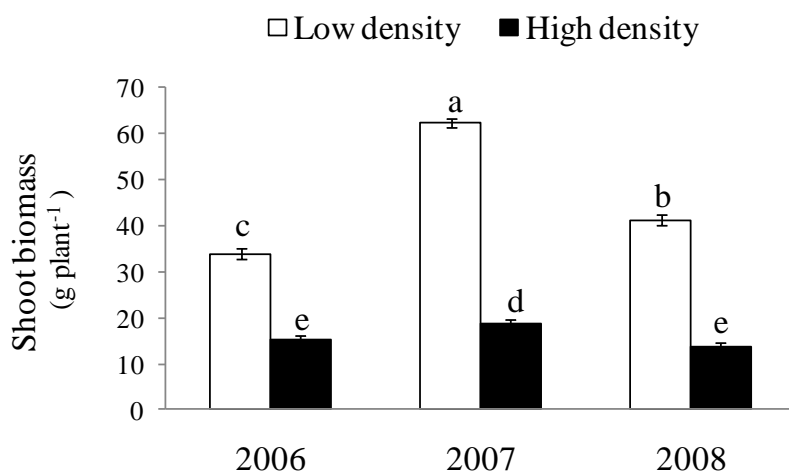


Figure 2. Means and standard errors of shoot biomass (g plant⁻¹) at low and high densities for 2006 – 2008. Different letters represent significant ($P < 0.05$) differences among year x density combinations.

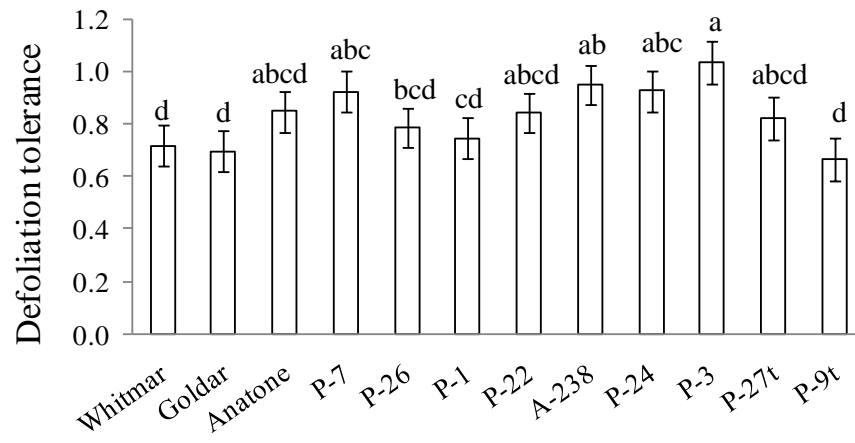


Figure 3. Means and standard errors for defoliation tolerance of 12 *P. spicata* populations across low and high densities calculated from three-year total biomass production. Different letters represent significant ($p < 0.05$) differences among populations.

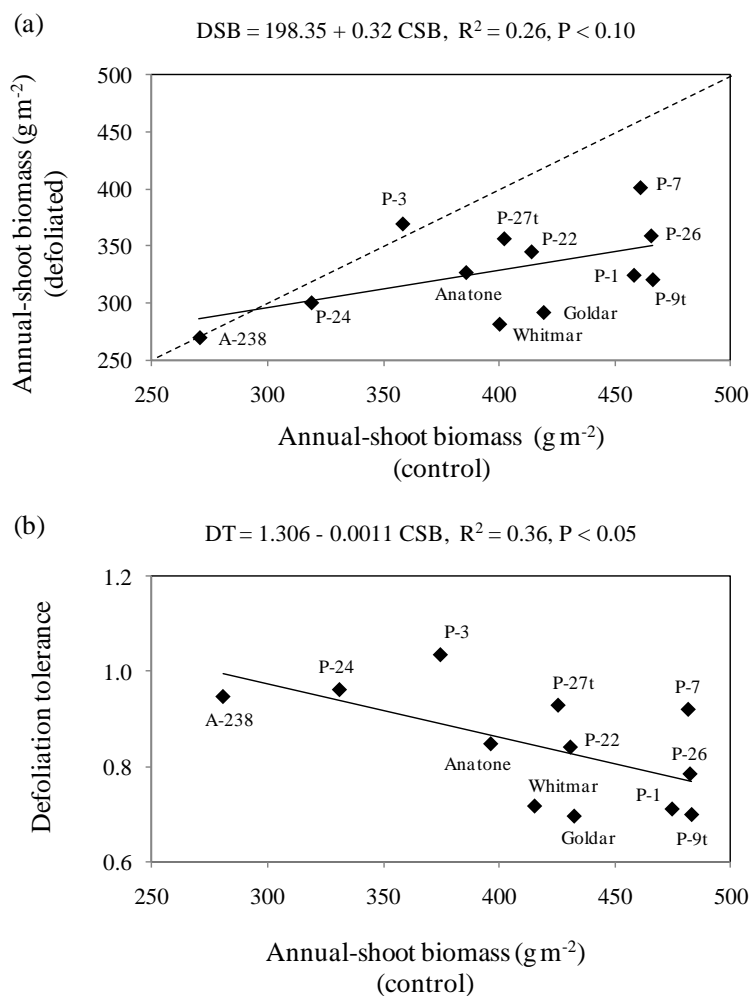


Figure 4. Linear regression of (a) three-year total defoliated shoot biomass (DSB) on control shoot biomass (CSB) and (b) defoliation tolerance (DT) on three-year total control shoot biomass.

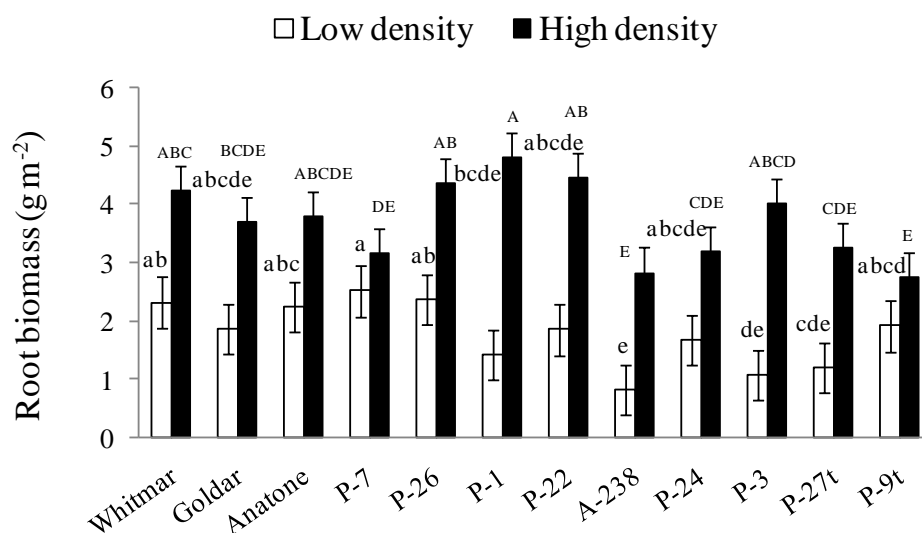


Figure 5. Means and standard errors of 12 *P. spicata* populations for root biomass in 2008 at high and low densities. Different letters represent significant ($P < 0.05$) differences among populations at low (lower case) and high (upper case) density treatments.

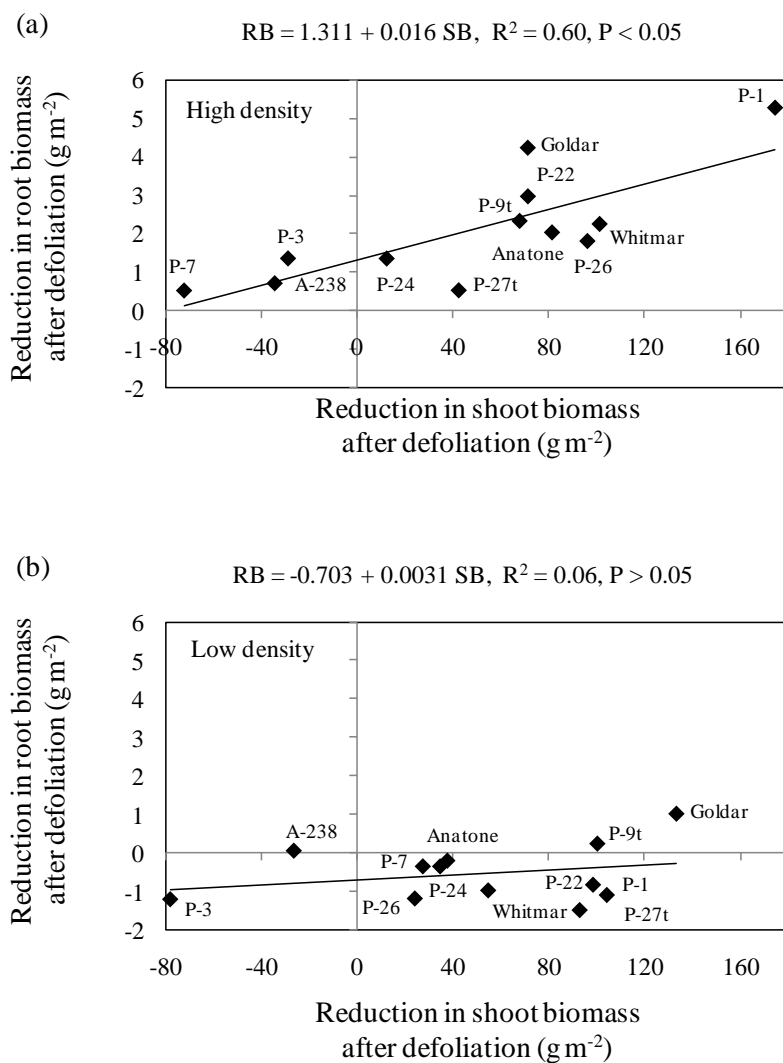


Figure 6. Linear regression of reduction in 2008 root biomass (RB) on reduction in 2008 shoot biomass (SB) for (a) high- and (b) low-density treatments.

CHAPTER 7

SYNTHESIS

Abstract

Over the past decade, increased interest has been expressed in restoring ecosystem processes on landscapes, as opposed to restoring biodiversity (Bradshaw 1996; Naeem 2006; Whisenant 1999). Consequently, the importance of functional plant traits for plant materials to be used in ecosystem restoration has become more widely recognized (Young 2005; Sheley et al. 2006). As ecological restoration typically involves active inputs to promote recovery and reconstruction of functional assemblages, understanding the physiological limitations of species and populations to stress and disturbance regimes becomes imperative. Sagebrush-steppe ecosystems of the North American Intermountain West that have been degraded by past overgrazing, weed invasions, and increased wildfire frequencies offer an ideal opportunity to address important eco-physiological questions (Ehleringer and Sandquist 2006; Young 2005).

In my research I addressed a few broader questions, e.g.,

- which seedling traits are associated with early germination,
- how may these traits contribute to seedling growth and establishment,
- which physiological traits may contribute to drought and defoliation tolerance
- do species or populations that vary for tolerance vary also in these traits, and
- what is the nature of physiological trade-offs among our traits of interest?

To achieve the ultimate goal of developing more effective restoration plant materials, my purpose was to improve the understanding of the functional ranges of two native perennial bunchgrasses, *Pseudoroegneria spicata* and *Elymus wawawaiensis*, regarding 1) seedling establishment, 2) drought tolerance, and 3) defoliation tolerance.

By comparing germination and growth of 23-day old seedlings of the two native bunchgrasses to the invasive alien species, *Bromus tectorum*, I found that the superiority of *B. tectorum* at the very-young seedling stage depends primarily on rapid germination and significant investments in root surface area (root:shoot length ratio), as opposed to above-ground investments (Chapter 2). On the other hand, *P. spicata* exhibited the highest seedling biomass among the three species. While greater biomass may indicate better establishment, *E. wawawaiensis*'s high specific leaf area (SLA) and specific root length (SRL), are commonly associated with faster growth. The intraspecific comparisons showed that populations with larger seeds had greater seedling biomass, while populations with smaller seeds were associated with higher SLA and SRL, traits that have been linked to faster growth (Poorter and Garnier 2007) (Chapter 3). In comparing interspecific and intraspecific variation for seedling traits, it was interesting to note that SLA was more different between species than among *P. spicata* populations (Chapter 2). On the other hand, SRL varied both across (Chapter 2) and within (Chapter 3) species, suggesting that SRL is more variable while SLA is less variable within these species.

In my study, by comparing *P. spicata* and *E. wawawaiensis* under drought stress, I found that the latter was relatively more drought tolerant than *P. spicata*. According to the eco-physiological literature, plants with greater stress tolerance display lesser growth potential. However, my greenhouse study showed that *E. wawawaiensis* was more

drought tolerant than *P. spicata*, despite the former's higher SLA and SRL. To infer to relative growth rate (RGR) in my study, I used SLA as its predictor (Poorter and Garnier 2007), and I found no evidence of a trade-off between stress tolerance and RGR (SLA) between these two species (Grime 2001). While the two species displayed similar water use efficiency (WUE) when water was abundant, *E. wawawaiensis* was more efficient in its water use when drought stress was imposed (Chapter 4). Water use efficiency is a trait commonly used as a selection criterion for superior drought tolerance. While, instantaneous WUE has been widely used for selecting drought-tolerant plant materials (Blum 1988), we measured WUE of productivity. Low water loss of *E. wawawaiensis* indicates that this species may also have high instantaneous WUE as well. More recent studies on grass architecture show that self-shading reduces water loss and increases water use efficiency (Ramirez et al. 2008). Hence, it might be reasonable to hypothesize that species with high SLA, which may generate greater foliage area, may also cause more self-shading to reduce water loss, increasing WUE. Similar to the findings of the germination studies (Chapters 2, 3), SLA differed between species, but not for populations within species, with the exception of one *P. spicata* population.

Based on *E. wawawaiensis*'s greater defoliated-to-control shoot-biomass ratio, I concluded that *E. wawawaiensis*, with its higher SLA, was more defoliation tolerant than *P. spicata* (Chapter 5). Under field conditions, SLA did not differ among populations within species, although both species were significantly different for SLA. In this study, I was also able to determine that plant defoliation tolerance is more uniform when all individuals were defoliated at a same phenological stage. My results suggest that, to select desirable species or populations for restoration purposes, it is important to apply

the defoliation treatment at the same phenological stage rather than on the same calendar date. Similar to drought response (Chapter 4), I found a positive relationship between SLA (Chapter 5) and defoliation tolerance, suggesting a lack of trade-off between relative growth and defoliation tolerance (Grime 2001). In spite of this, I also found that a species with low SLA produced greater shoot biomass in the absence of defoliation and was less defoliation tolerant, suggesting a trade-off between growth and defoliation tolerance.

I compared *P. spicata* populations for defoliation tolerance under low and high plant densities (Chapter 6). I found that populations that were less productive in the absence of defoliation were more defoliation tolerant, supporting the presence of an evolutionary trade-off between defoliation tolerance and productivity. Anatone and P-7, two recent plant material releases that are the most commonly used for restoration purposes today, were less sensitive to defoliation than the older *P. spicata* plant materials, ‘Whitmar’ and ‘Goldar’, despite the fact that the newer plant materials were not selected for grazing tolerance.

Goldar and Anatone *P. spicata* and ‘Secar’ *E. wawawaiensis* were common to all my studies. My results justify the use of Secar *E. wawawaiensis* as a high-performing surrogate for *P. spicata* in restoration because of its greater drought and defoliation tolerance capabilities. Anatone was the *P. spicata* population most comparable to *E. wawawaiensis* for tolerance to stress, particularly defoliation. Although Goldar displayed similar defoliation tolerance to Anatone when individual plants were defoliated, at high densities, more typical of rangeland conditions, Goldar was more sensitive to defoliation.

My research provides baseline data for the range of morphological and physiological traits that may be used to identify plant materials with superior seedling

establishment and drought and defoliation tolerance. Additionally, there are other aspects of this research that may warrant further exploration:

1. 'Discovery', the recent *E. wawawaiensis* release, is an intended alternative to Secar (Jones 2008). In my study, Discovery and Secar showed similar defoliation tolerance, although this evaluation was made on individual plants. Tolerance ability under rangeland conditions, where plants occur at greater densities and/or are exposed to severe droughts, may pose a greater challenge. Future studies evaluating Discovery's seedling characteristics and tolerance capabilities in comparison with Secar are essential for restoration applications.
2. For restoration applications, it is important to consider redundancy within species and functional groups. This is because some species and populations may play a minimal role in ecosystem function until environmental conditions change, upon which they may confer flexibility in response to rapid events (Walker 1992; Walker 1993; Naeem 2006). For the restoration of Intermountain West ecosystems, 'regional seeding mixtures' may be particularly valuable (Ehleringer and Sandquist 2006). My study shows that significant functional variation exists among *P. spicata* populations. For example, *P. spicata* populations like P-3, A-238, and P-24 (Chapter 6) exhibited less productivity, yet higher defoliation tolerance, than more productive populations. Combining such populations within a seeded plant community may increase its resilience and stability of the community. Research focusing on such questions is highly desirable.
3. Collectively, my research has demonstrated that SLA may be an important trait for selection of plant materials for restoration purposes (also see Rebetzke et

al. 2004). Vigor of germinating seedlings (young-seedling productivity) is critical for successful seedling establishment, and improved seedling vigor in crop plants, e.g., wheat and barley, has often been associated with higher SLA (Rebetzke et al. 2004). Genotypic differences in SLA can provide plant material development programs with the necessary variation to improve early seedling vigor (Rebetzke and Richards 1999). Recent findings for these grain crops show that genetic variation in SLA is due to additive genetic effects with small-to-moderate narrow-sense heritability values (Rebetzke et al. 2004).

Furthermore, degraded and restored plant communities are evolving under current environmental conditions (Leger 2008). Given such a context, it is important to understand combinations of functional traits that will lead to better and more stable species coexistence.

References

- BLUM, A. 1988. Plant breeding for stress environments. Boca Raton, Florida, USA: CRC Press. 223 p.
- BRADSHAW, A.D. 1996. Underlying principles of restoration. *Canadian Journal of Fish and Aquatic Science* 53: 3-9.
- EHLERINGER, J.R. AND R.D. SANDQUIST. 2006. Ecophysiological constraints on plant responses in a restoration setting. In: D.A. Falk, M.A. Palmer, and J.B. Zelder [EDS]. *Foundation of restoration ecology*. Island Press, Washington, WA, USA. p 42-58.
- GRIME, J.P. 2001. Plant strategies, vegetation processes, and ecosystem properties. New York, USA: John Wiley & Sons. 460 p.

- JONES, T.A. 2008. Notice of release of 'Discovery' Snake River wheatgrass. *Native Plants Journal* 9: 99-101.
- LEDGER, E.A. 2008. The adaptive value of remnant native plants in invaded communities: an example for the native species. *Ecological Applications* 18:1226-1235.
- NAEEM, S. 2006. Biodiversity and ecosystem functioning in restored ecosystems: extracting principles for a synthetic perspective. In: D.A. Falk, M.A. Palmer, and J.B. Zelder [EDS]. *Foundation of restoration ecology*. Island Press, Washington, WA., USA. p 210-237.
- POORTER, H., AND E. GARNIER. 2007. Ecological significance of inherent variation in relative growth rate and its components. In: F.L. Pugnaire and F. Valladares F [EDS]. *Functional plant ecology*. CRC Press, Boca Raton, FL., USA. p 67-100.
- RAMIREZ, D.A., F. VALLADARAS, A. BLASCO, AND J. BELLOT. 2008. Effect of tussock size and soil water content on whole plant gas exchange in *Stipa tenacissima* L.: extrapolating from the leaf versus modelling crown architecture. *Environmental and Experimental Botany* 62: 376-388.
- REBETZKE, G.J., AND R.A. RICHARDS. 1999. Genetic improvement of early vigor in wheat. *Australian Journal of Agricultural Research* 50: 291-301.
- REBETZKE, G.J., T.L. BOTWRIGHT, C.S. MOORE, R.A. RICHARDS, AND A.G. CONDON. 2004. Genotypic variation for specific leaf area for genetic improvement of early vigor in wheat. *Field Crops Research* 88: 179-189.
- SHELEY, R.L., J.M. MANGOLD, AND J.L. ANDERSON. 2006. Potential for successional theory to guide restoration of invasive-plant-dominated rangeland. *Ecological Monographs* 76: 365-379.

WALKER, B. 1992. Biodiversity and ecological redundancy. *Conservation Biology* 6: 18-23.

WALKER, B. 1993. Rangeland ecology: understanding and managing change. *Ambio* 22: 80-87.

WHISENANT, S.G. 1999. Repairing damaged wildlands: a process-oriented, landscape-scale approach. Cambridge, UK: Cambridge University Press. 312 p.

YOUNG, T.P. 2005. The ecology of restoration: historical links, emerging issues and unexplored realms. *Ecology Letters* 8: 662-673.

APPENDIX

Table A1. Average-monthly and total-annual precipitation (mm) for 2006, 2007, 2008, and 2009 recorded at Logan Experimental Farm, Utah.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2006	89.14	26.92	100.07	50.29	24.64	18.8	7.36	24.13	74.67	37.59	35.82	13.21	502.64
2007	4.82	48.51	37.85	21.59	31.24	18.8	16.26	14.22	29.21	32.76	9.41	50.03	314.7
2008	60.45	41.65	27.18	17.02	46.74	20.57	1.78	28.44	34.04	23.61	25.65	41.91	369.04
2009	57.14	45.95	54.35	65.54	48.76	56.37	--	--	--	--	--	--	--

CURRICULUM VITAE

Jayanti Ray-Mukherjee

9064 Collins Ave.

Apt # 12, Surfside

Miami, FL 33154

Ph: 786-512-3632

e-mail: jayanti.rm@gmail.com

Academic training:

2005 – 2010:	Ph.D.	Utah State University (USU)	Ecology
<i>Supervisors:</i>		<i>Dr. Peter B. Adler (USU) & Dr. Thomas A. Jones (USDA)</i>	
1993 – 95:	MS	Viswa-Bharati University, INDIA	Botany
1990 – 93:	BS	Viswa-Bharati University, INDIA	Botany

Academic and Research Experience:

2005-2009	Utah State University/USDA, USA	Research Assistant
2003-2004	Ben-Gurion University, ISRAEL (volunteer)	Research technician
2000-2001	Wildlife Institute of India, INDIA (World Bank)	Project Associate
1998-2000	Wildlife Institute of India, INDIA	Senior Research Fellow
1996-1998	Wildlife Institute of India, INDIA	Junior Research Fellow

Awards and Scholarships:

2007	Ecology Center (USU) Travel Award (\$500).
2007	Graduate Student Senate (USU) Travel Grant (\$200)
2005	Dean's Honor List (USU)
2003	Best paper and presentation MASHAV International Course, Israel.
2003	MASHAV Fellowship, Ministry of External affairs, Govt. of Israel.
1991-1993	National Scholarship, Govt. of India (for undergraduate studies).

Publications:

Mukherjee, J. R., Chelladurai, V., Ronald, J., Rawat, G. S., Mani, P and M. A. Huffman
(*in press*). Do animals eat what we do?: ethnobotanical and ecological

- observations on humans and animals of Mundanthurai range, Tamil Nadu, India. In: C. P. Kala (ed.). Medicinal plants and sustainable development. *In press*.
- Zaady, E., **Mukherjee, J.**, Godonu, K.J., Kraaij, T., Mwangi, M., Oguzoglu, I., Sarig, S. (2006). Small-scale landscape patchiness affects soil characteristics in Negev Highlands (Abstract). *Israel Journal of Ecology and Evolution* 52: 203-204.
- Ray, J.*** and G.S. Rawat (2002). Patterns of plant species diversity and endemism in Kalakad-Mundanthurai Tiger Reserve, Tamilnadu. In: A.P. Das (ed.). Perspectives of Plant Biodiversity. Pp. 559-557. Shiva Offset Printers, Dehra Dun.
- Ray, J.*** and Rawat, G. S. (2001). Patterns of Plant Species Diversity and Endemism in Kalakad – Mundanthurai Tiger Reserve, Tamil Nadu. Proceedings on the National Seminar on Plant Biodiversity – Systematics, Conservation and Ethno botany, IAAT, Cochin, India.
- Ray, J.***, Joshua, J., Ronald, J. (2000). Sighting of Barking Deer in Kalakad-Mundanthurai Tiger Reserve, Tirunelveli, Tamil Nadu. *Journal of Bombay Natural History Society* 97: 139 – 140.
- Ray, J.***, Chelladurai, V., Mani, P*, Rawat, G. S. (1998). Observations on some Medicinal Plants of Mundanthurai Range in the Kalakad-Mundanthurai Tiger Reserve, Tamil Nadu. Proceedings of the International Conservation Conference 1998, Tiruvananthapuram, India.
- *Maiden name

Manuscripts in review and in preparation:

- Mukherjee, J. R.**, Jones, T. A., Adler, P. B. and T. A. Monaco, T. A. Drought tolerance in two perennial bunchgrasses for restoration in the Intermountain West, U.S.A. *In review (Plant Ecology)*
- Mukherjee, J. R.**, Jones, T. A., Adler, P. B. and T. A. Monaco, T. A. Immature seedling growth of two North American native perennial bunchgrasses and the invasive grass *Bromus tectorum*.
- Mukherjee, J. R.**, Jones, T. A., Adler, P. B. and T. A. Monaco, T. A. Seed mass, growth rates, and functional traits of nine populations of *Pseudoroegneria spicata* under contrasting temperatures.
- Mukherjee, J. R.**, Jones, T. A., Adler, P. B. and T. A. Monaco, T. A. Coping with herbivory: relative responses of morphological traits of two semiarid perennial bunchgrasses under two clipping regimes.
- Mukherjee, J. R.**, Jones, T.A. and T. A. Monaco. Differential defoliation response of *Pseudoroegneria spicata* genotypes at two densities.

Popular Articles:

Mukherjee, J. R. (2005). Biodiversity Hotspot Highlight: Western Ghats. *Biological Conservation Newsletter*.

Seminars, Workshops, Presentations and Courses:

2009 Odum Conference, Albany, NY. “Seedling traits of native perennials vs. invasive annuals: a case study in the Intermountain West”.

2008 Graduate Student Symposium, Utah State University, UT. “Restoration using native grasses: the importance of plant traits”.

2007 Ecological Society of America, San Jose, CA. “Effects of water frequency on individual plant performance: Implications for restoration”.

2003 Ben-Gurion University, Israel. “Long – term of Human Activities Affecting Soil Characteristics in Hyper – arid Ecosystems”. Best paper and presentation award.

2000 Indian Association of Angiosperm Taxonomy, Siliguri, WB, India. “Richness and endemism of Kalakad-Mundanthurai Tiger Reserve”.

1998 International Conservation Conference on Plant biodiversity and Conservation. Tropical Botanical Garden and Research Institute, Thiruvananthapuram, India.

Additional professional experience:

- Presider at “Phenotypic Plasticity: Plants” session of Ecological Society of America Annual Meeting, August 2007